dutch®

Recognizing Advanced Patterns with DUTCH

Mark Newman, Precision Analytical

Why Are We Here?

If you can Interpret the DUTCH Test and integrate with standard lab testing, you can help patients with more competence and confidence

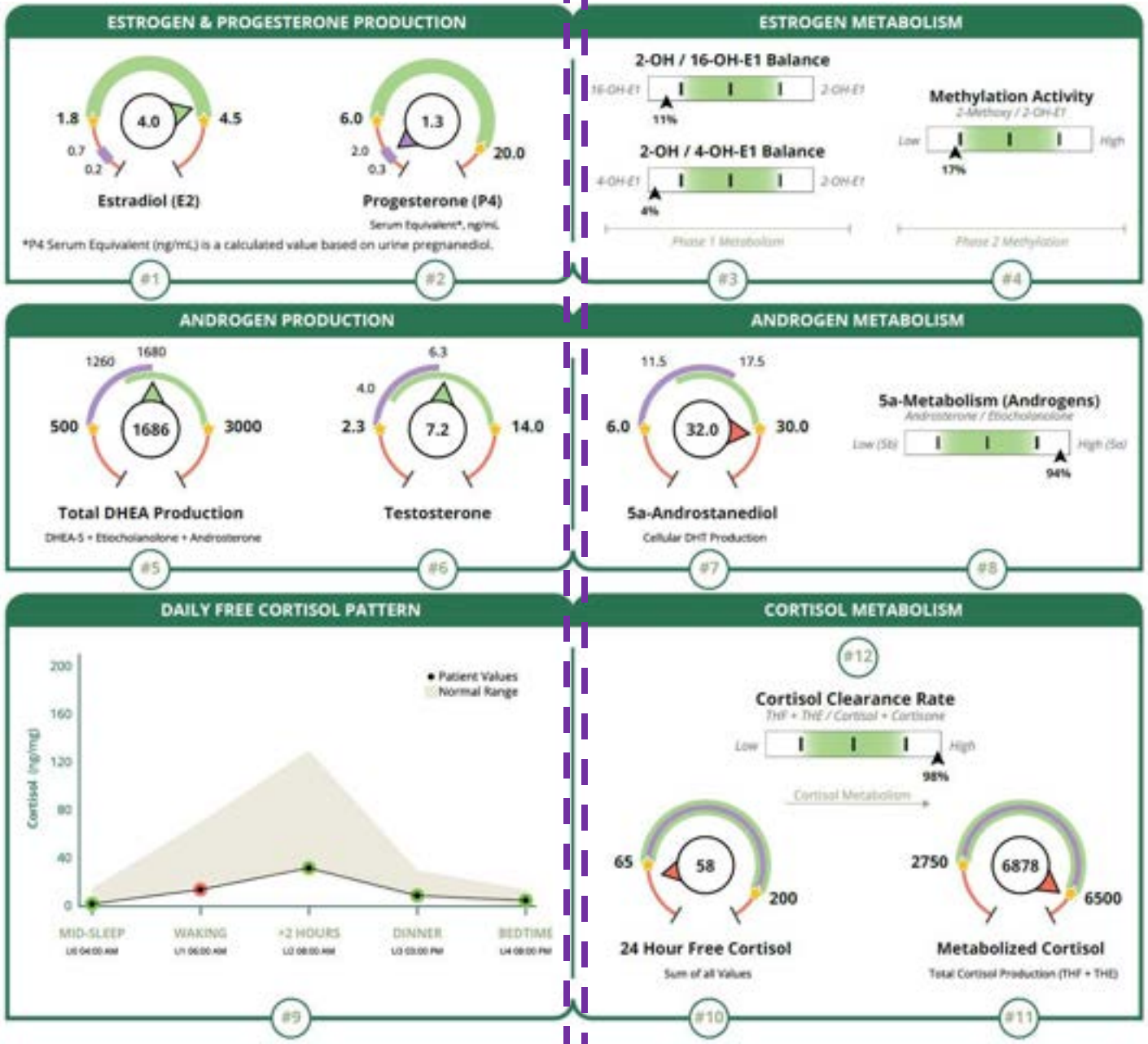
Why Are We Here Today?

- Move beyond the DUTCH Test Summary Page
- Dig deeper into the DUTCH Test with Advanced Insights
- Understand common caveats to interpretation

Estrogen Story

Androgen Story

Cortisol Story



Hormone Production

Hormone Metabolism

Hormone Testing Fundamentals

- How Lab Testing Can Lie to You
- DUTCH Distinctives – methods you can trust!
- Understanding free and metabolized cortisol

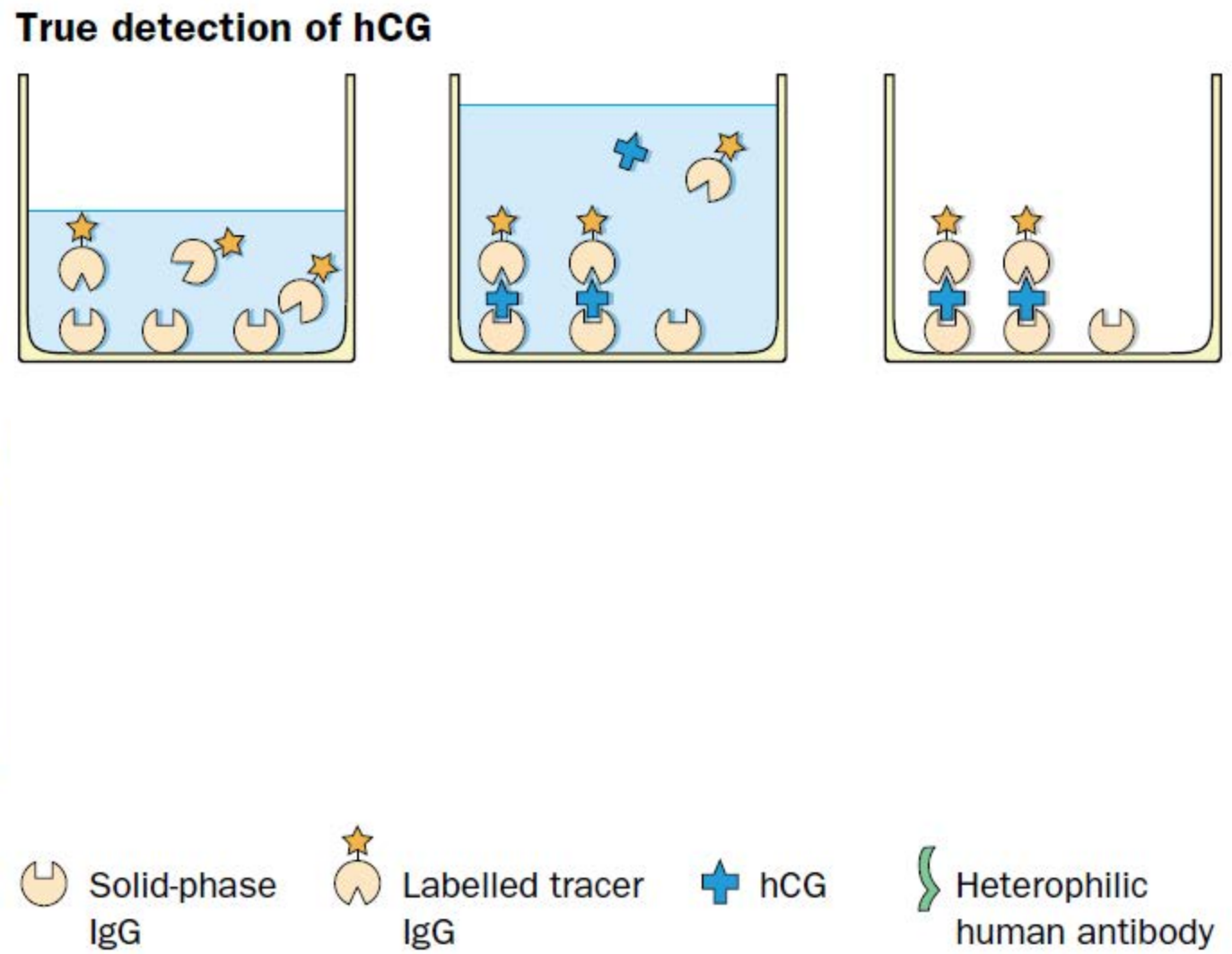
Labs are Useful Tools, Not infallible Truth Tellers

To serve patients well, we need to have perspective on the value and limitations of labs, including their ability to lie to us!

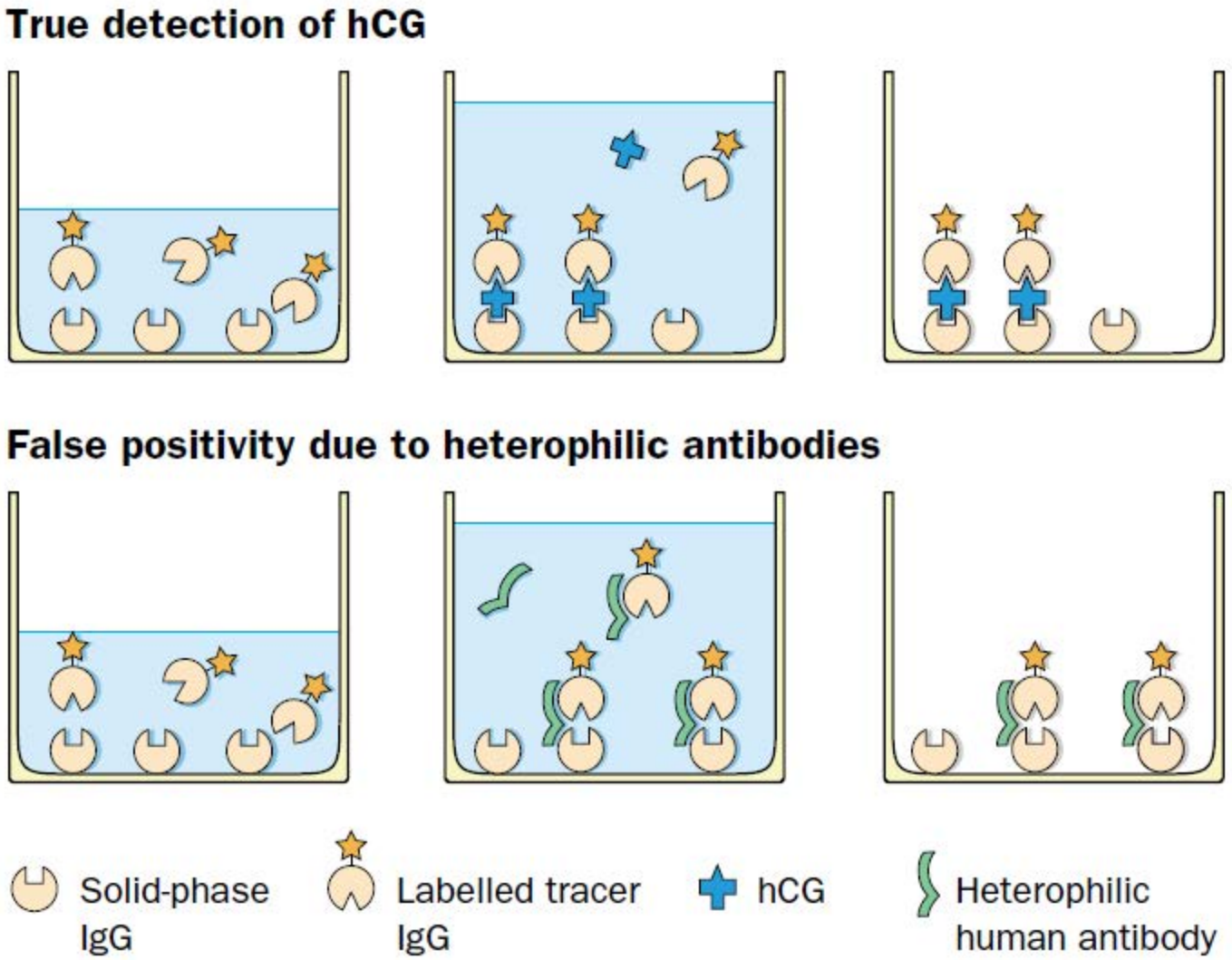
How Can a Lab Test Lie to You?

Measure something inaccurately

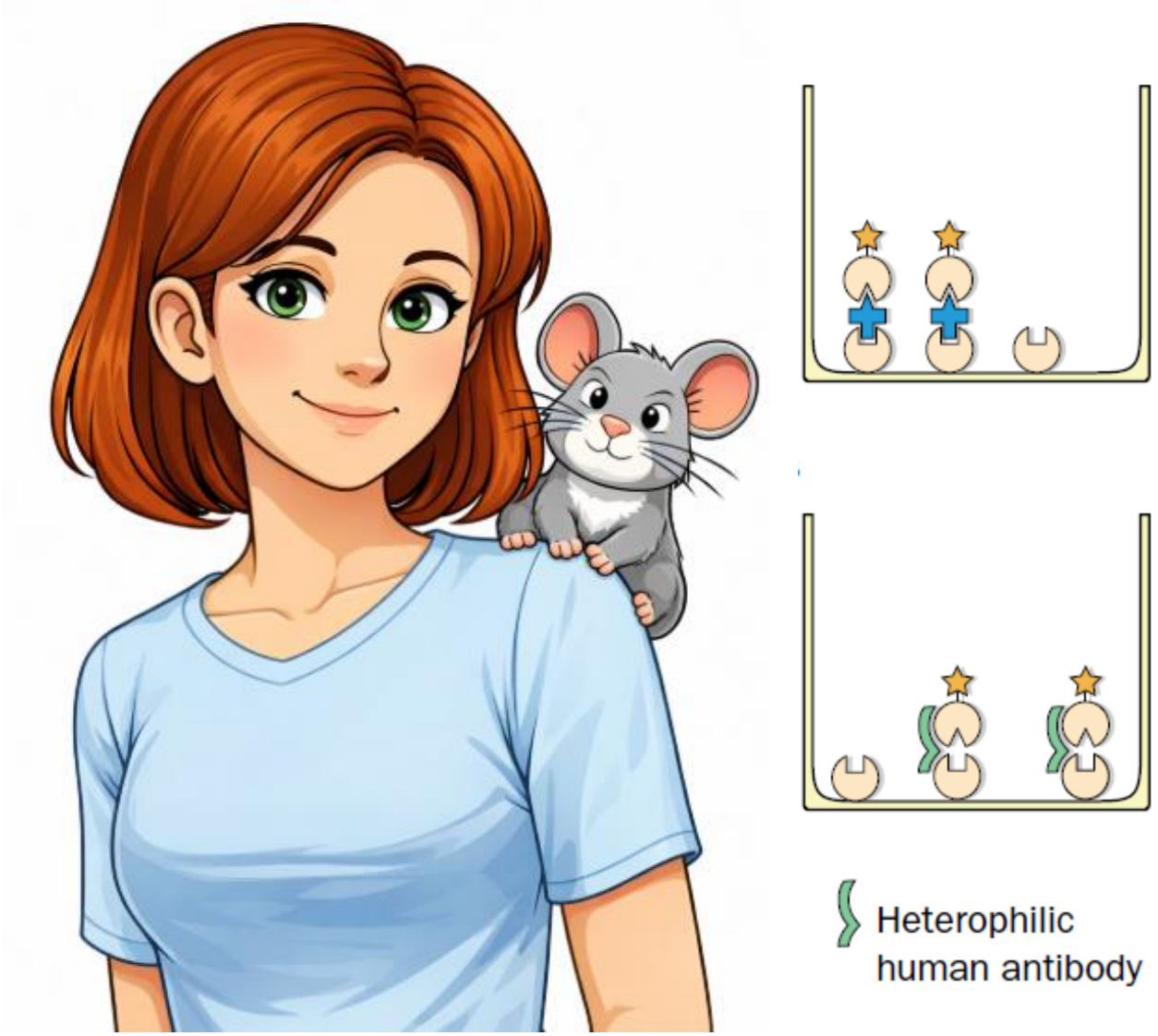
An example of how a lab test can REALLY lie to you



An example of how a lab test can REALLY lie to you



An example of how a lab test can REALLY lie to you



Consequences of a “bad” lab test

Patient	Age (years)	Number of pregnancies	Parity	Reason for hCG test	Surgical treatment	Chemotherapy
1	36	1	0	Incidental	D&C, laparoscopy, TAH	Methotrexate, EMAC, vincristine
2	23	1	0	Menstrual irregularity	D&C, laparoscopy, TAH, thoracotomy	Methotrexate, actinomycin D, EMAC, vincristine
3	26	1	0	Vaginal bleeding	D&C, laparoscopy, TAH	Methotrexate
4	40	3	3	Abdominal pain	D&C, laparoscopy, TAH, BSO	..
5	24	1	0	Menstrual irregularity	D&C, laparoscopy	Methotrexate, actinomycin D
6	36	2	2	Incidental	D&C, laparoscopy, BSO	Methotrexate
7	28	2	2	Abdominal pain	D&C, laparsocopy	Methotrexate
8	28	4	4	Incidental	D&C, laparoscopy	..
9	22	1	0	Incidental	D&C	..
10	42	2	2	Incidental	..	..
11	25	3	1	Incidental	D&C	..
12	25	Not known		Incidental	D&C	..

BSO=bilateral salpingo-oophorectomy; D&C=dilatation and curettage; EMAC=combination of etoposide, methotrexate, actinomycin D, and cyclophosphamide; TAH=total abdominal hysterectomy; Incidental=part of routine check-up, or before unrelated surgery or radiography.

Table 1: **Summary of case histories**

How Can a Lab Test Lie to You?

- Measure something inaccurately (this is why we use LC-MS/MS!)
- Primary marker may be insufficient

AACE Clinical Case Rep. 10 (2024) 206–209

Case Report

Exaggerated Increases in the Serum Cortisol Level in a Woman Following Oral Contraceptive Treatment

How Can a Lab Test Lie to You?

- Measure something inaccurately
- Primary marker may be insufficient

AACE Clinical Case Rep. 10 (2024) 206–209

Case Report: A 50-year-old woman presented with extreme morning hypercortisolemia—cortisol levels of 61 and 55 mcg/dL (4 and 3 months before presentation, respectively; normal range, 8-25 mcg/dL)—found during workup of mildly increased white cell counts. The morning cortisol level had been 10 mcg/dL after administration of 1-mg dexamethasone. The 24-hour urine free cortisol level had been normal and only slightly increased after correction by creatinine.

How Can a Lab Test Lie to You?

- Measure something inaccurately
- Primary marker may be insufficient
- Timing, collection issues
- Contamination

- What about specifically for DUTCH?

Urine Melatonin Marker Correlates with Gold Standard Testing

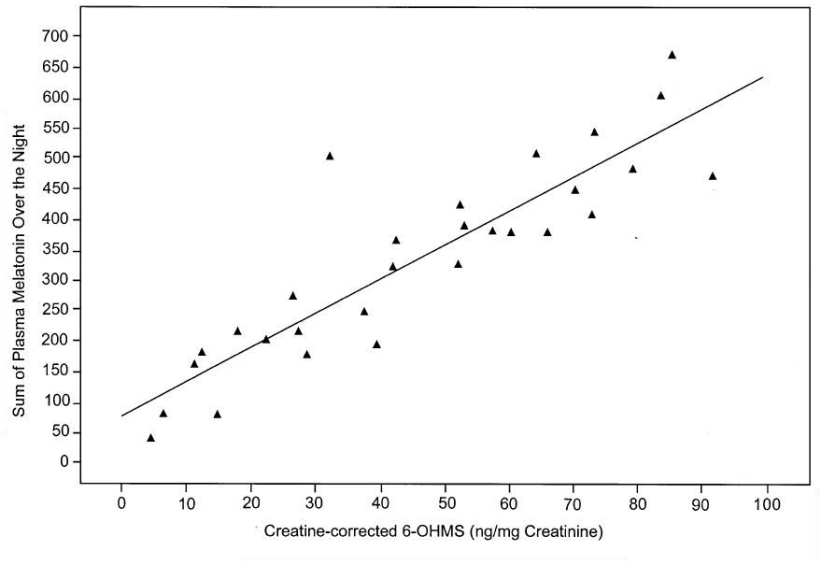


Fig. 3. Morning urinary 6-OHMS (ng/mg creatinine) was highly correlated (r = 0.89) with the AUC for nocturnal plasma melatonin (pg/mL) in this sample of 29 healthy 40–70-yr-old women.

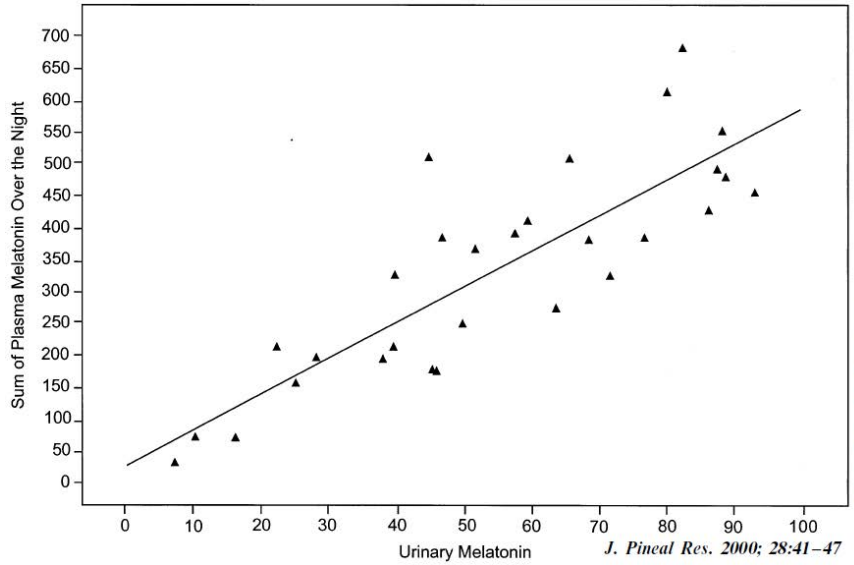
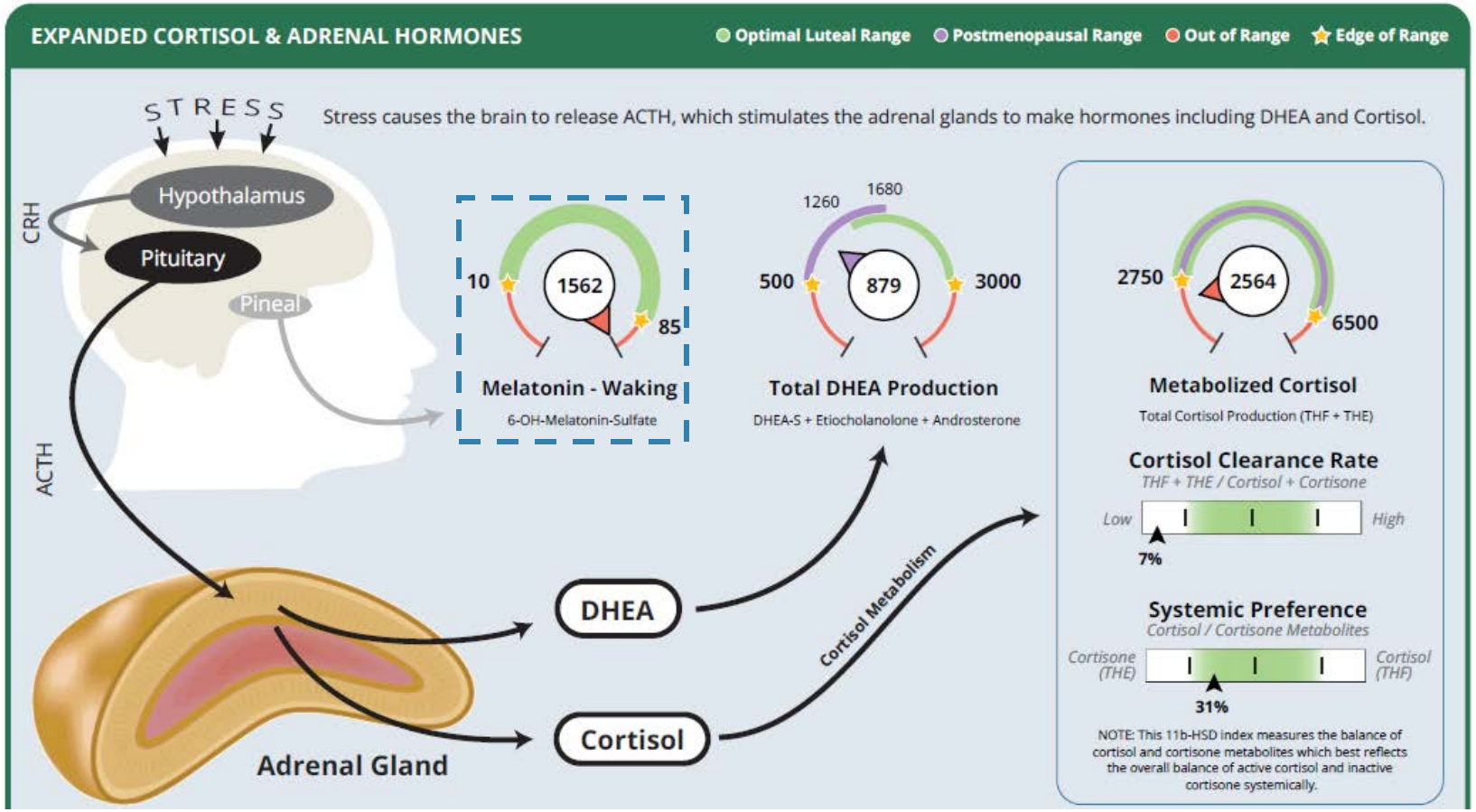


Fig. 4. Urinary melatonin (pg/mL) in morning samples correlated highly (r = 0.83) with AUC for nocturnal plasma melatonin (pg/mL) in this sample of 29 healthy 40–70-yr-old women.

How Can a DUTCH Test Lie to You?

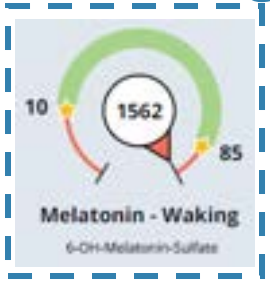
- Oral supplementation (beware melatonin, DHEA, E2, etc.)



How Can a DUTCH Test Lie to You?

- Oral supplementation (beware melatonin, DHEA, E2, etc.)

Not meaningful!



(Oral)
Melatonin → Melatonin
(tissue)

Melatonin
(in the gut)

6OH-Melatonin-S + 6OH-Melatonin-S
(not meaningful) (meaningful)

How Can a DUTCH Test Lie to You?

- Oral supplementation (beware melatonin, DHEA, E2, etc.)
- Timing, collection issues
- Contamination
- Creatinine anomalies
- Phase 2 metabolism anomalies

How Can a DUTCH Test Lie to You?

- Oral supplementation (beware melatonin, DHEA, E2, etc.)
- **Timing, collection issues**
- Contamination
- Creatinine anomalies
- Phase 2 metabolism anomalies

Common Timing Issues with DUTCH

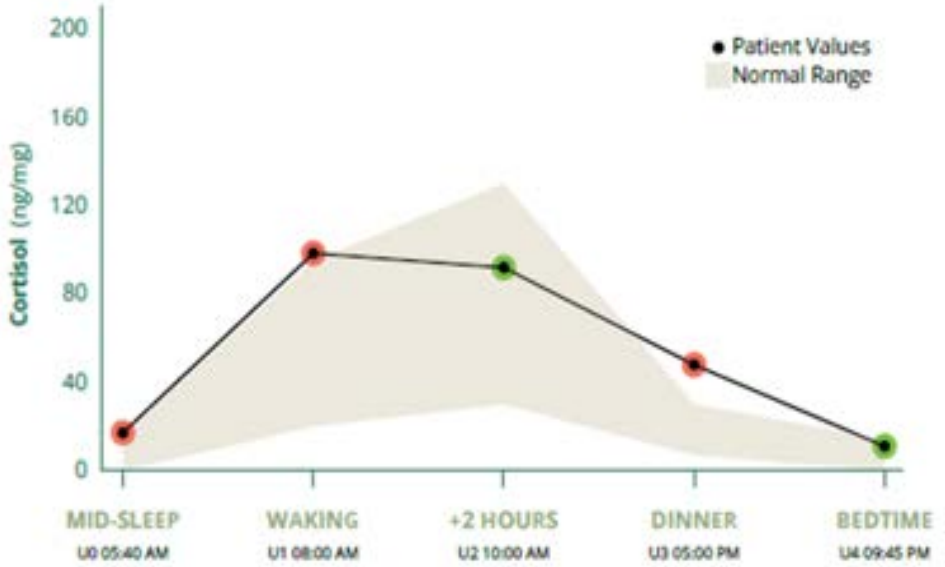
- Life events (surgery, migraine, etc.)
- Time of cycle
- Cycling but not bleeding (partial hysterectomy, ablation)
- HRT
- Birth Control cessation
- Cortisol timing can be “off” for several reasons

Common Timing Issues with DUTCH

- Life events (surgery, migraine, etc.)
- Time of cycle
- Cycling but not bleeding (partial hysterectomy, ablation)
- HRT
- Birth Control cessation
- **Cortisol timing** can be “off” for several reasons

Common Timing Issues with DUTCH - Cortisol

- Transitory life events (surgery, migraine, etc.)
- Shift work
- CAR before rising
- Atypical sleep disturbances



How Can a DUTCH Test Lie to You?

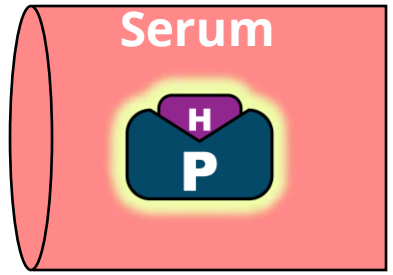
- Oral supplementation (beware melatonin, DHEA, E2, etc.)
- Timing, collection issues
- **Contamination**
- Creatinine anomalies
- Phase 2 metabolism anomalies

How Can Contamination Impact DUTCH

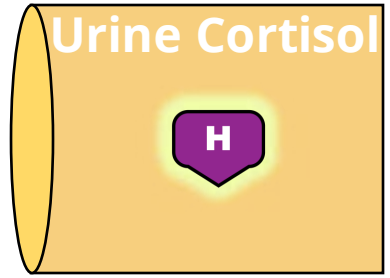
- Topical/Vaginal Hormones – a problem solved with clever lab methodology

What's in urine? Mostly conjugated E2, T, etc.

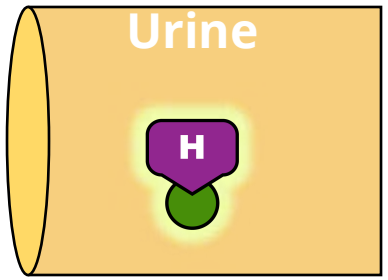
Protein-bound (SHBG, Albumin)



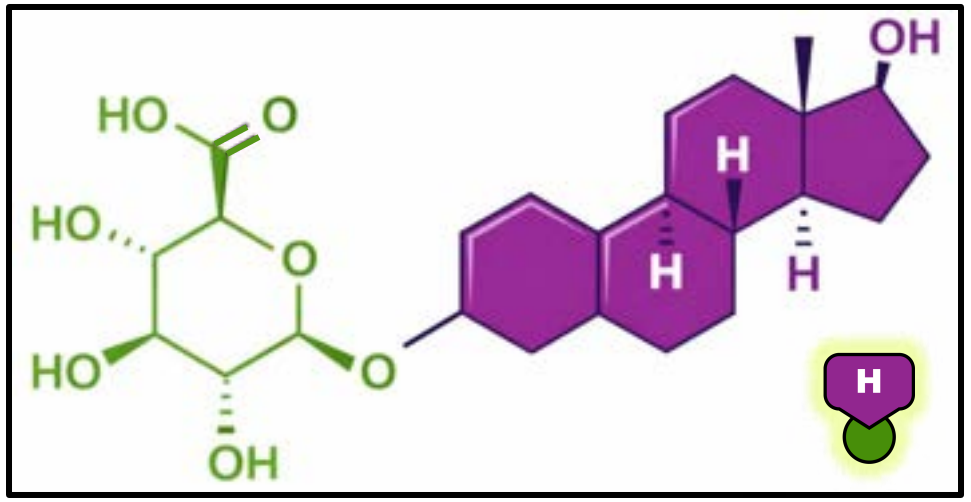
Free (unbound) Hormone



Hormone Conjugate (water-soluble)

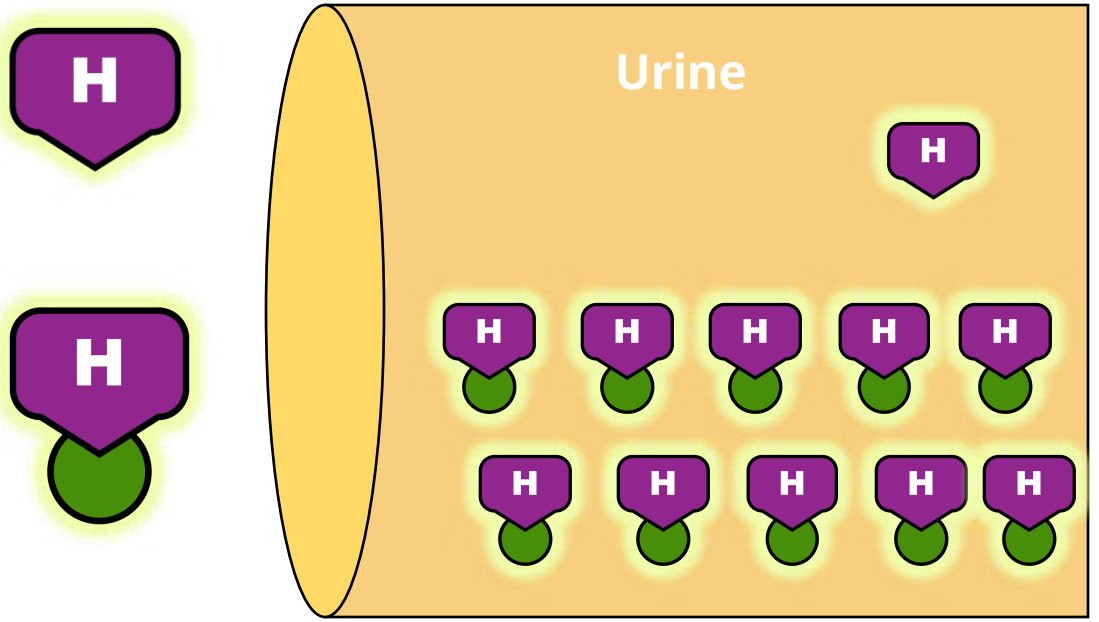


What's in urine? Mostly conjugated E2, T, etc.



Free E2 (naturally <<10%)

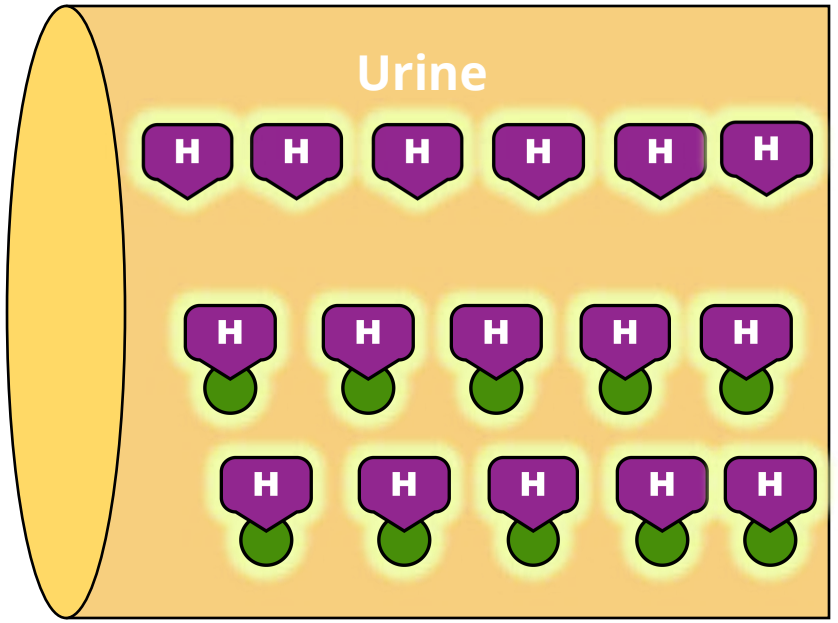
E2-Glucuronide (water-soluble)



What if the urine is contaminated by HRT?

Free E2 (with contamination)

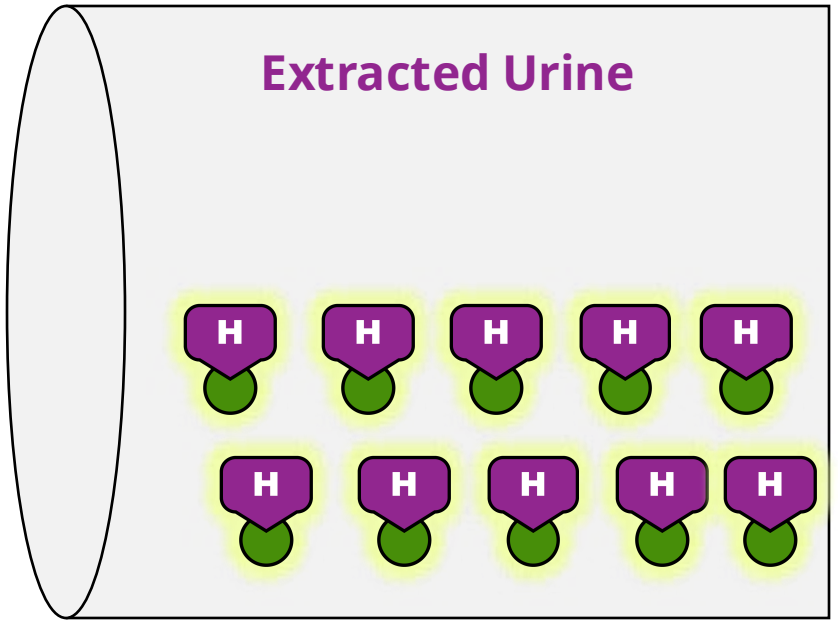
E2-Glucuronide (water-soluble)



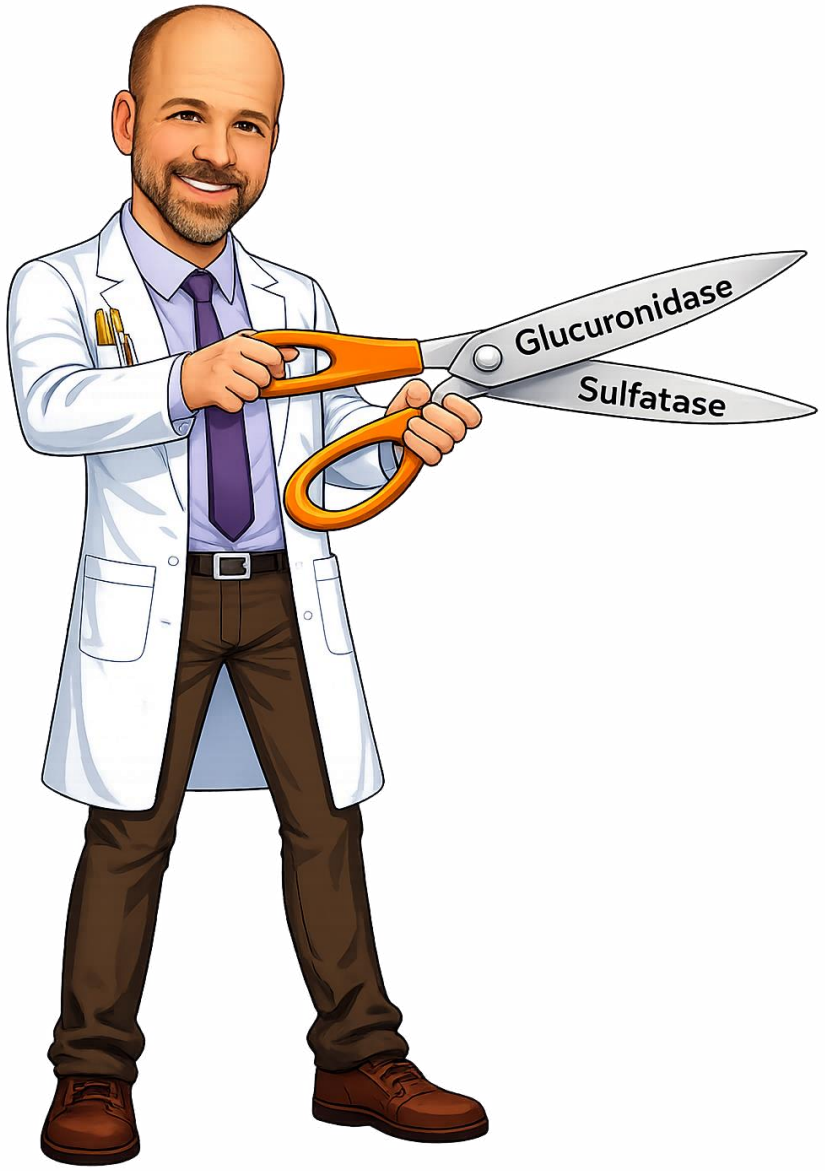
What if the urine is contaminated by HRT?

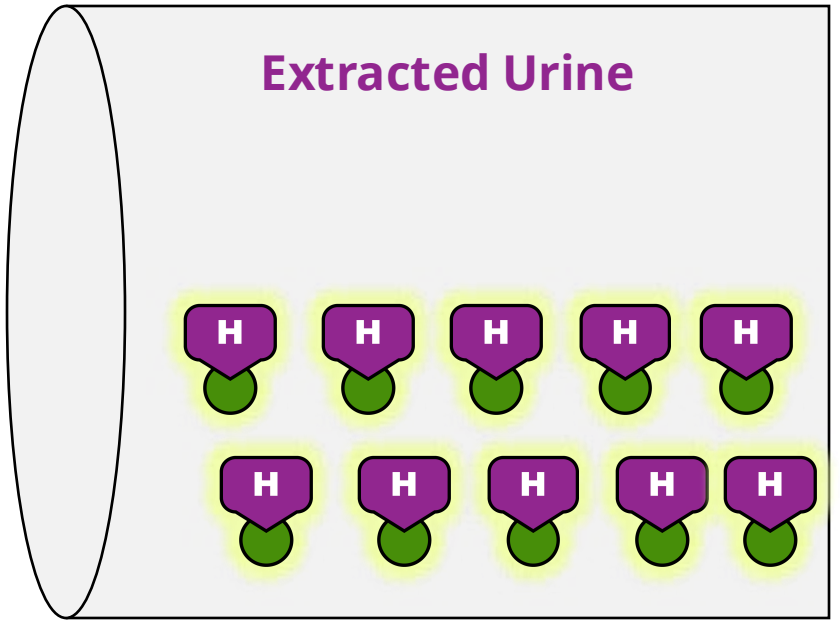
Free E2 (contamination removed)

E2-Glucuronide (water-soluble)

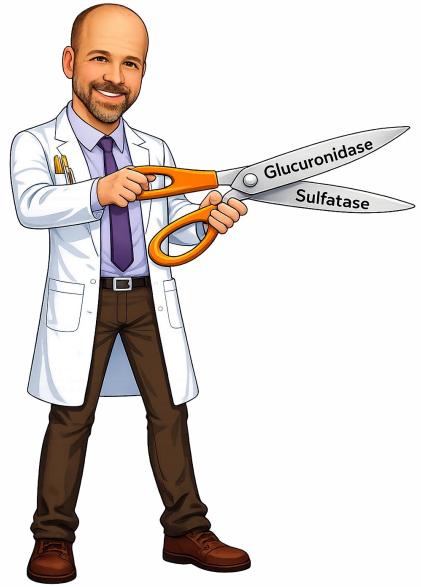


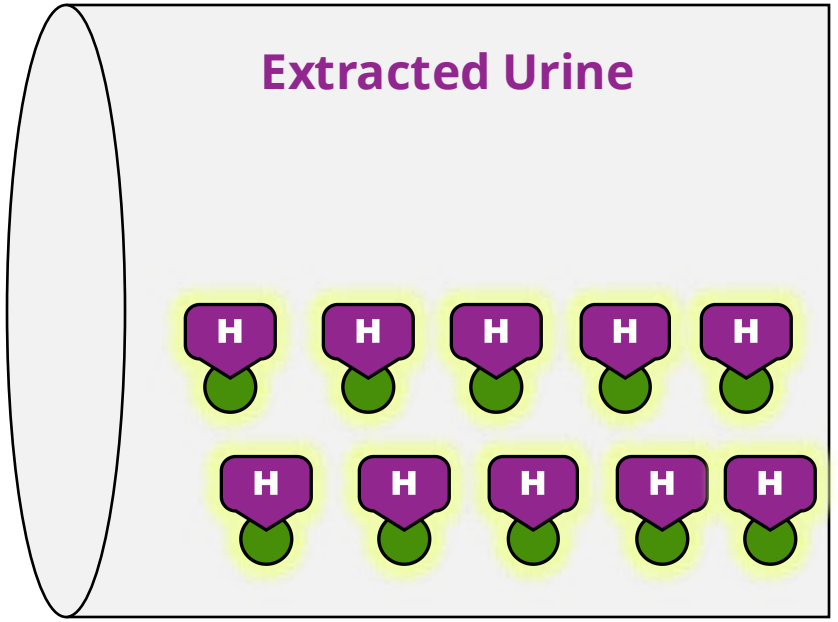
What if the urine is contaminated by HRT?





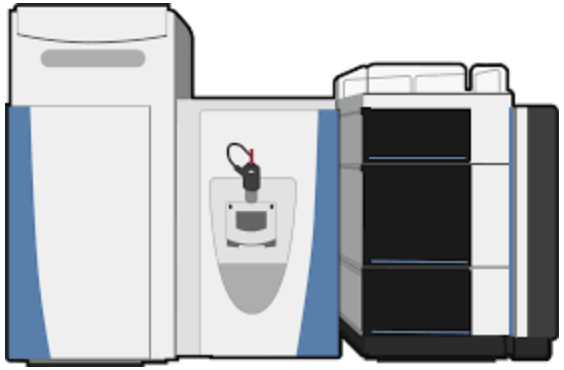
What if the urine is contaminated by HRT?



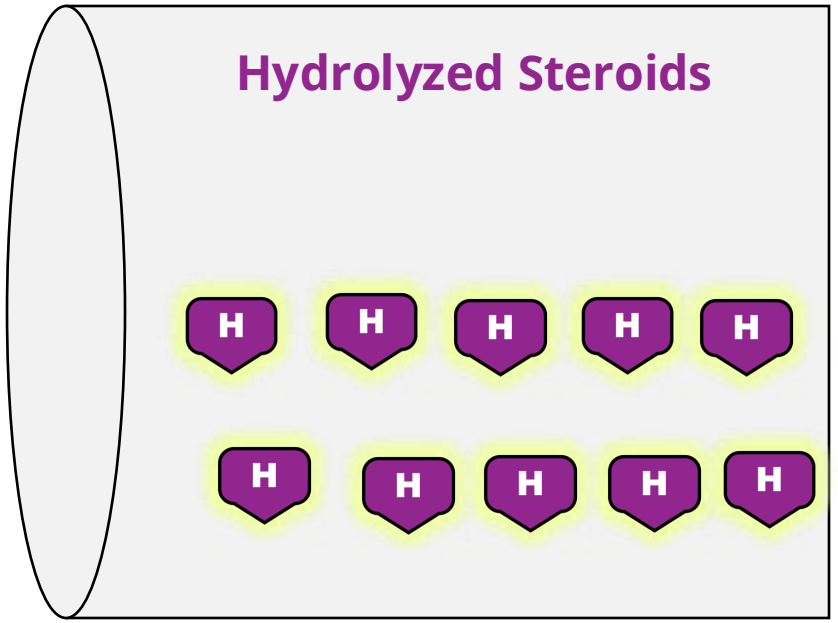


Urine samples, extracted, derivatized, and ready for LC-MS/MS

LC-MS/MS

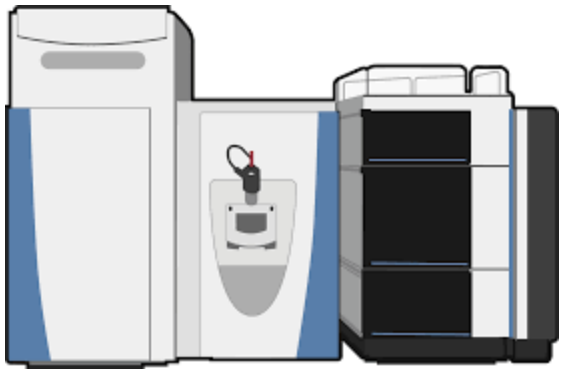


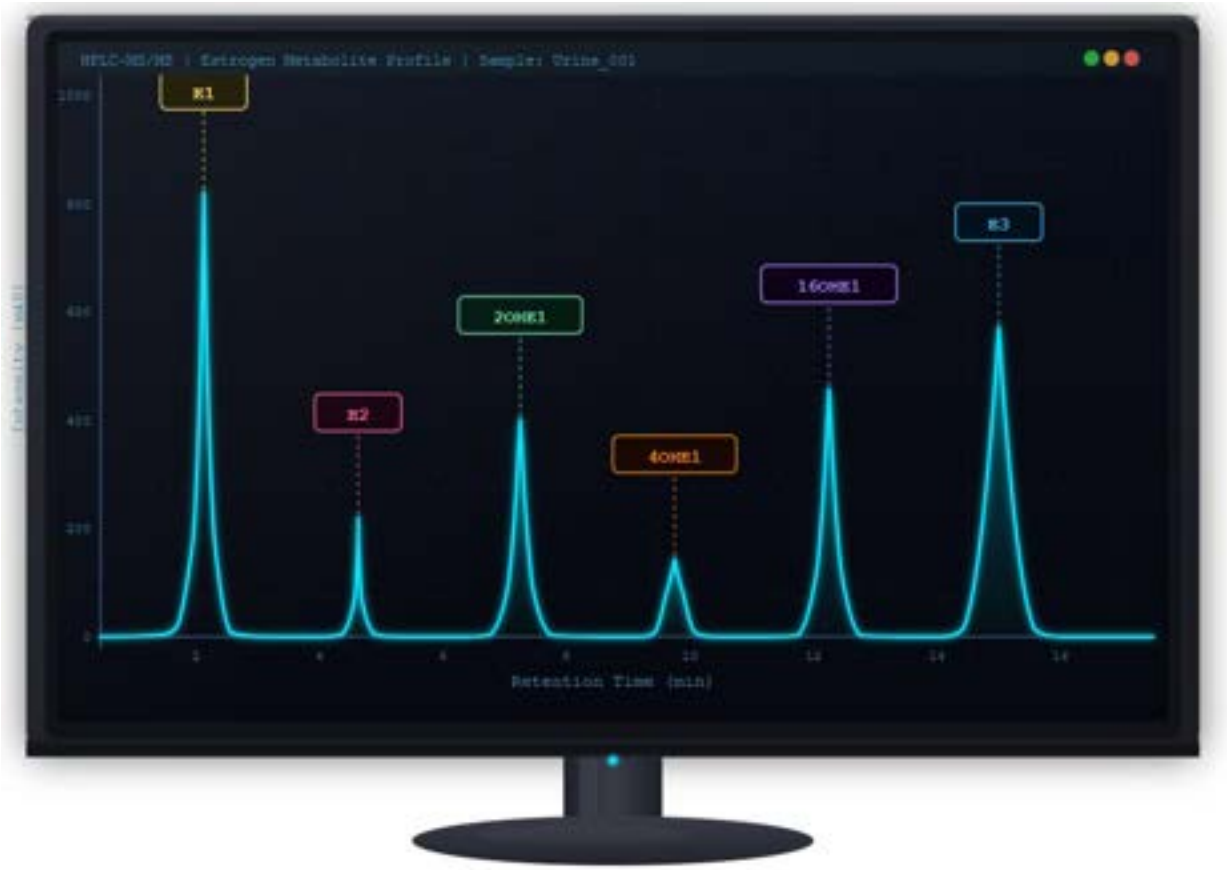
Free E2
(ready for analysis)



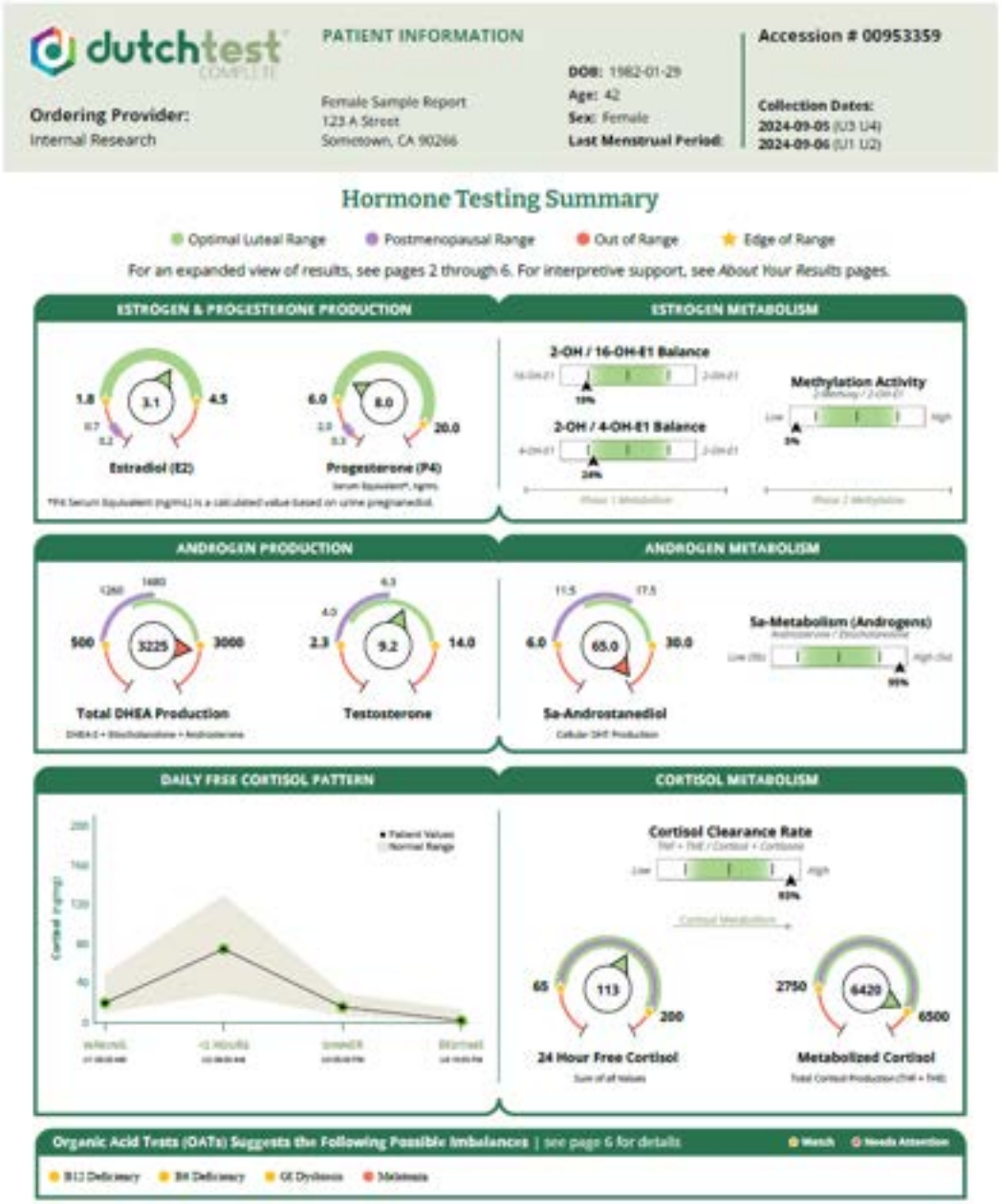
Urine samples, extracted, derivatized, and ready for LC-MS/MS

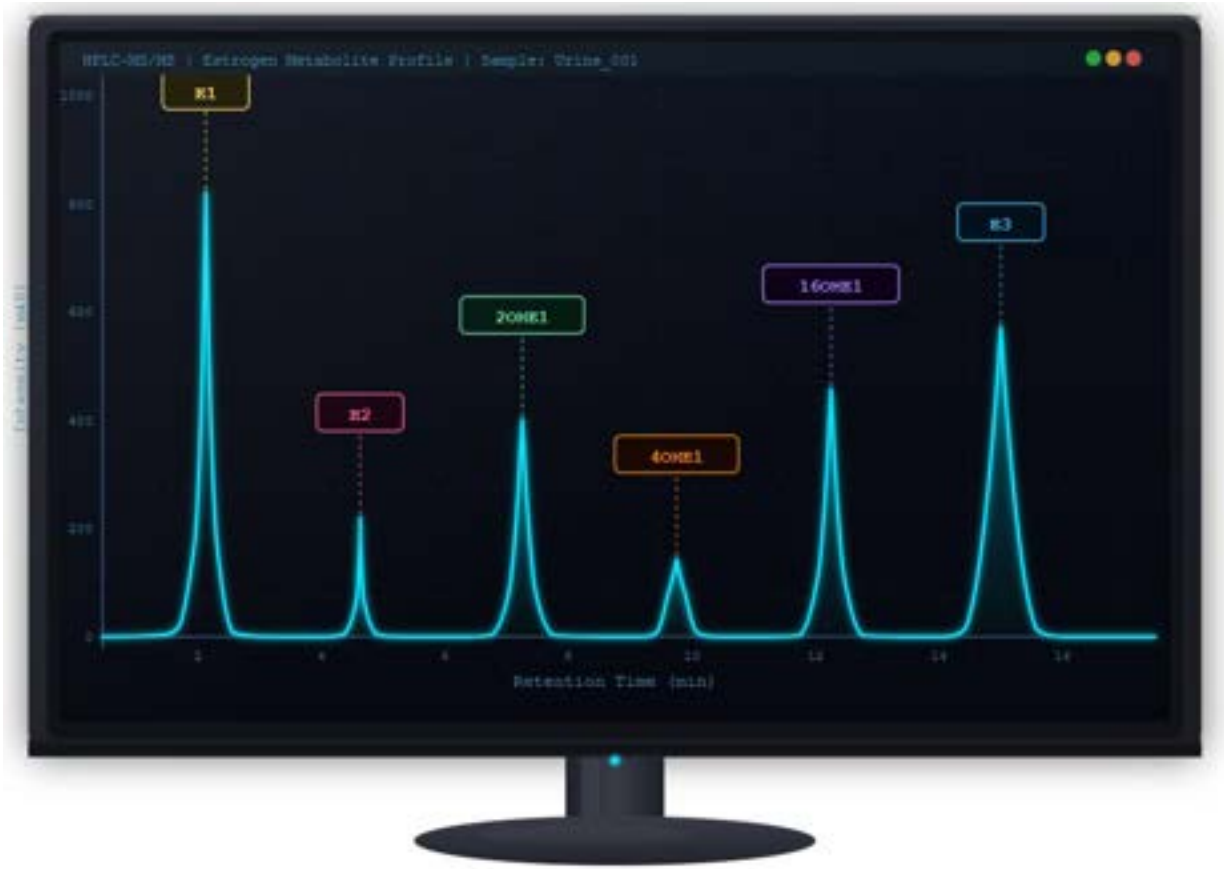
LC-MS/MS



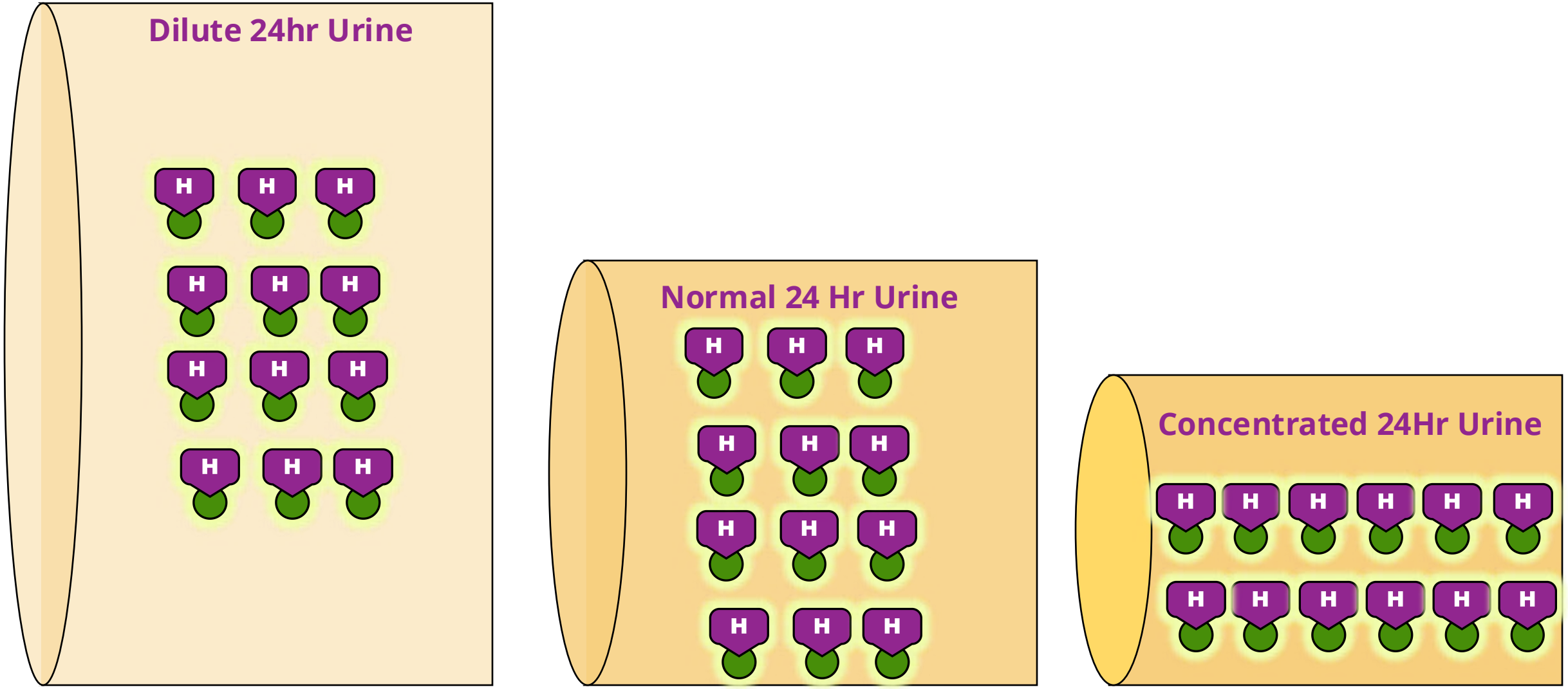


Urine samples, extracted, derivatized, and ready for LC-MS/MS

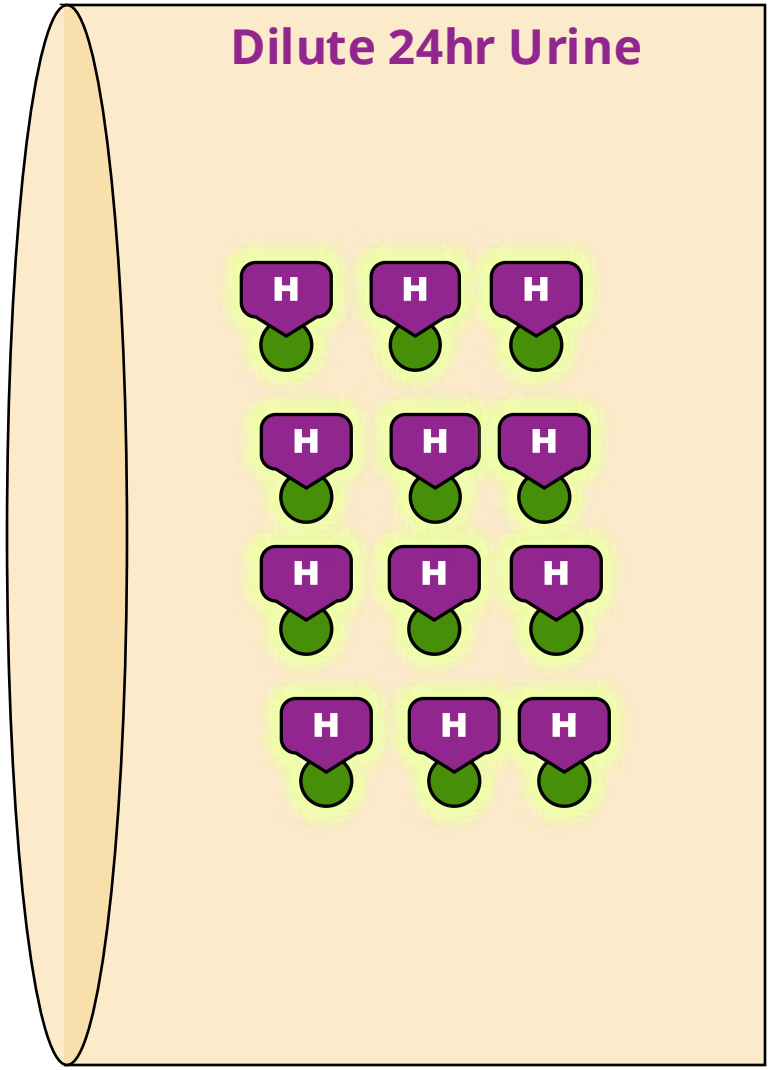


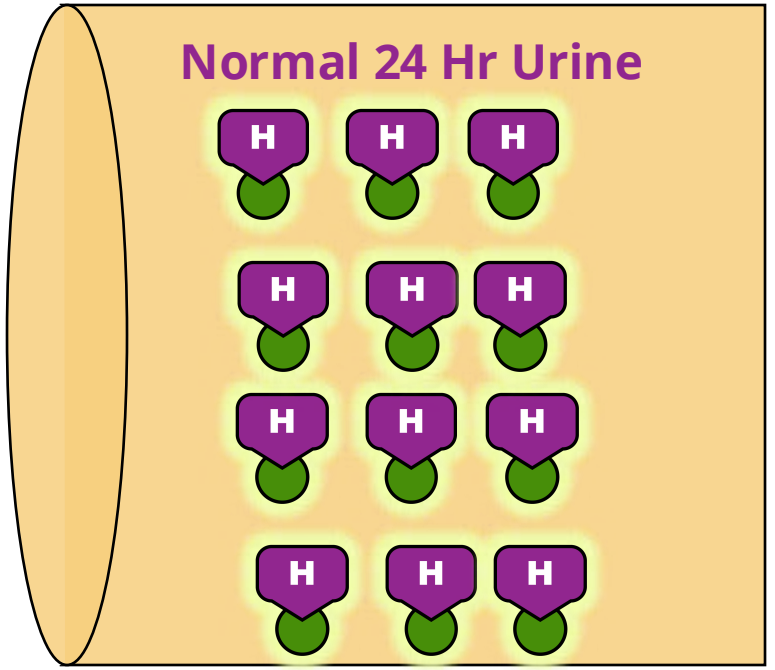


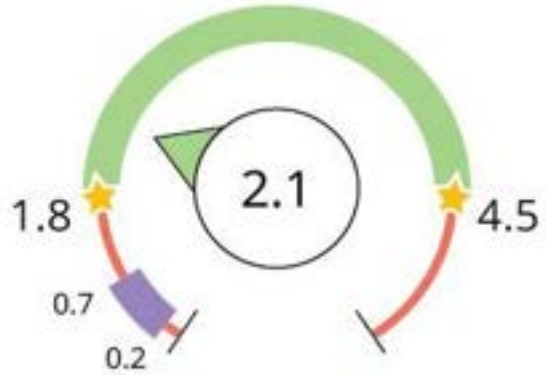
How does this work?



How does this work?



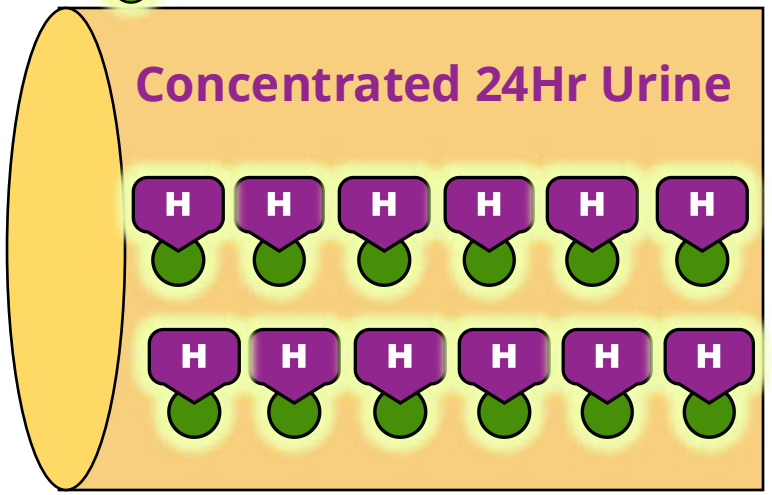




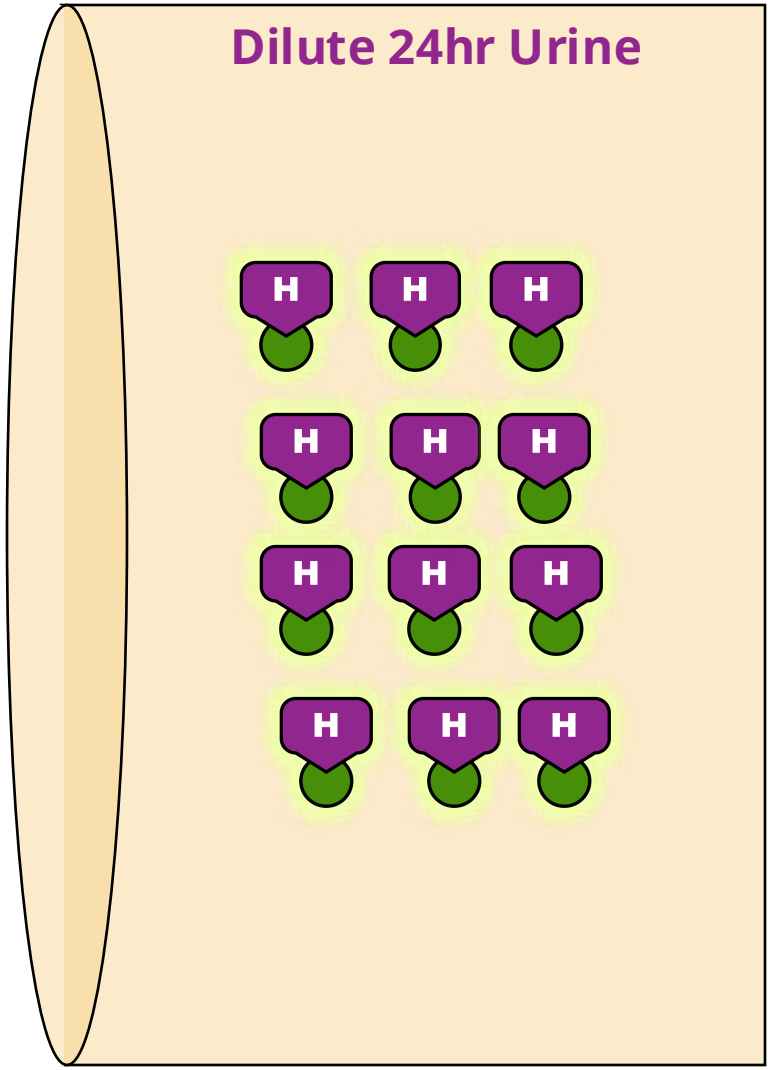
H = 4.2ng/mL

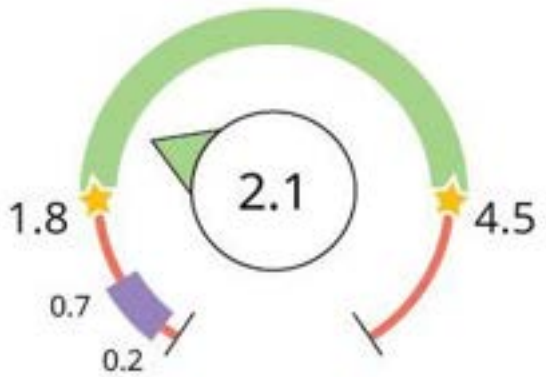
Creatinine = 2.0mg/mL

H = **2.1ng/mg** Creatinine



How does this work?



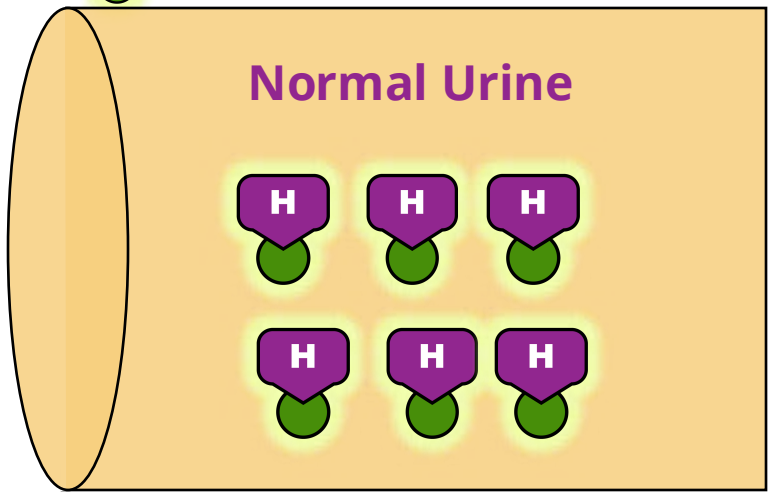


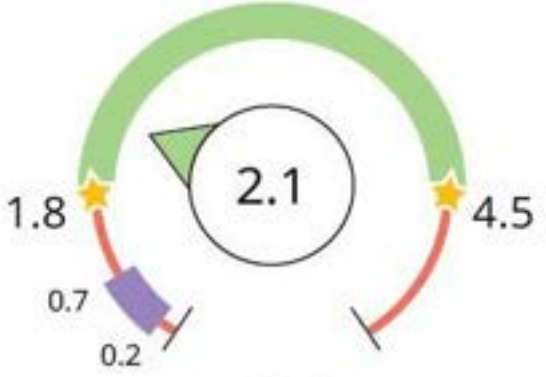
Estradiol (E2)

H = 2.1ng/mL

Creatinine = 1.0mg/mL

H = **2.1ng/mg** Creatinine



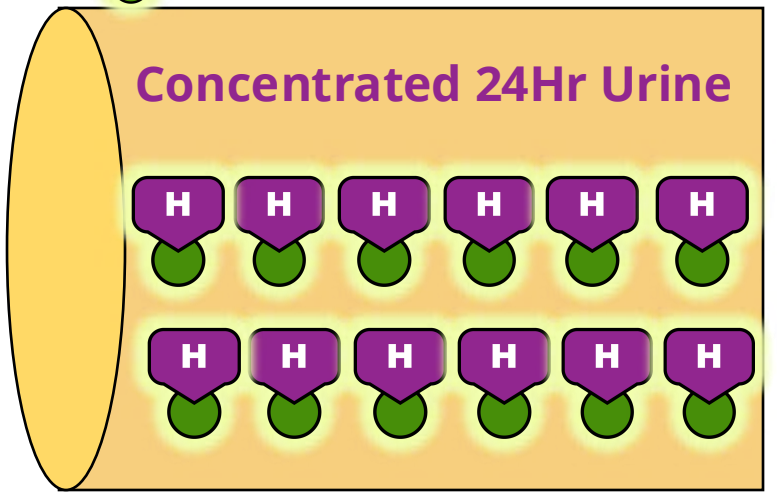


Estradiol (E2)

H = 4.2ng/mL

Creatinine = 2.0mg/mL

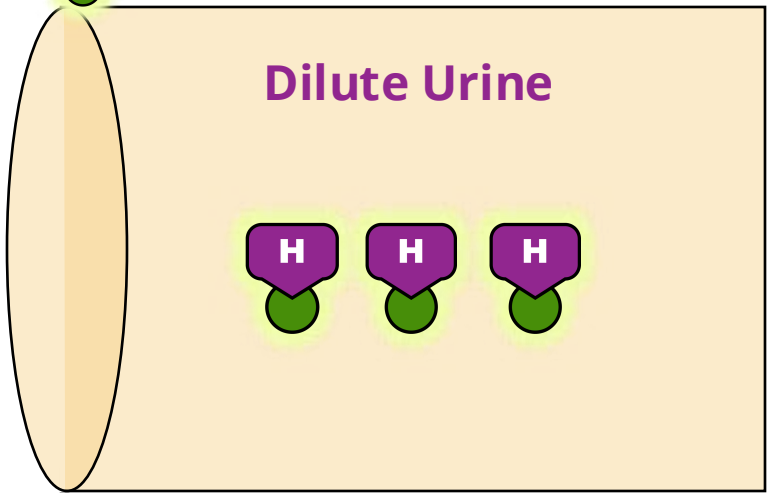
H = **2.1ng/mg** Creatinine

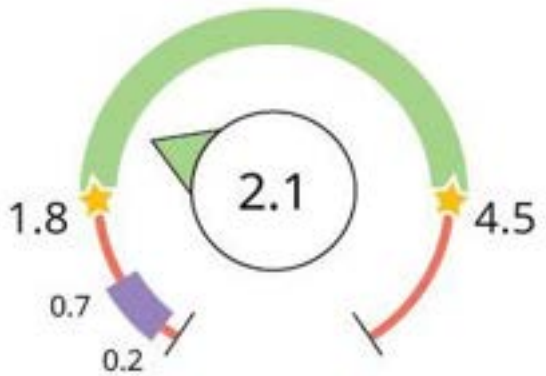


How does this work?

Estradiol (E2)

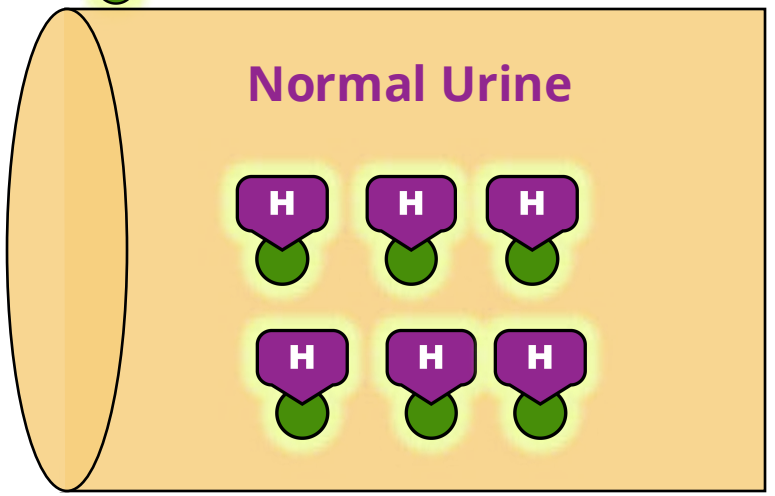
H = 1.05ng/mL
Creatinine = 0.5mg/mL
H = **2.1ng/mg** Creatinine

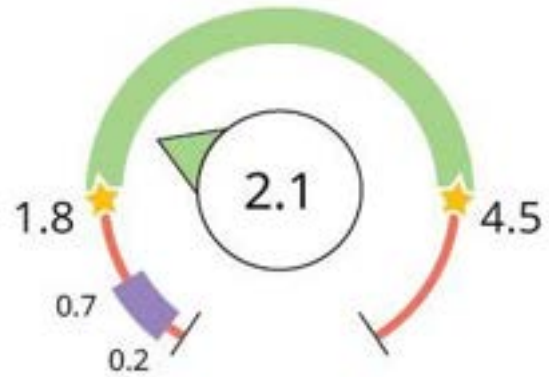




Estradiol (E2)

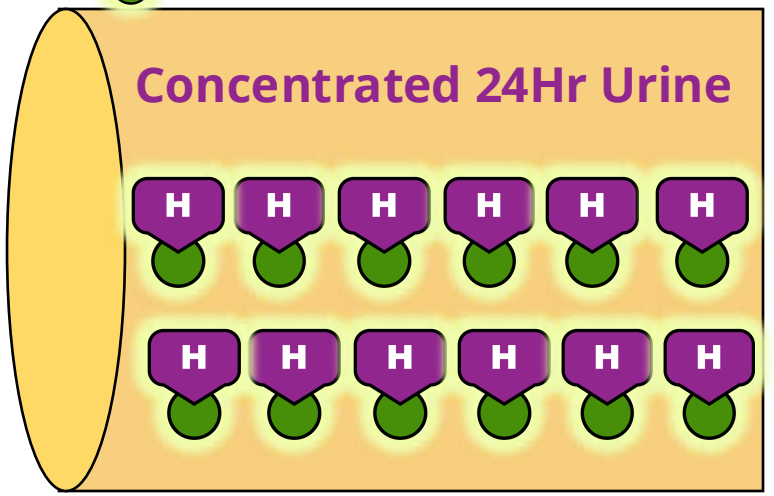
H = 2.1ng/mL
Creatinine = 1.0mg/mL
H = **2.1ng/mg** Creatinine



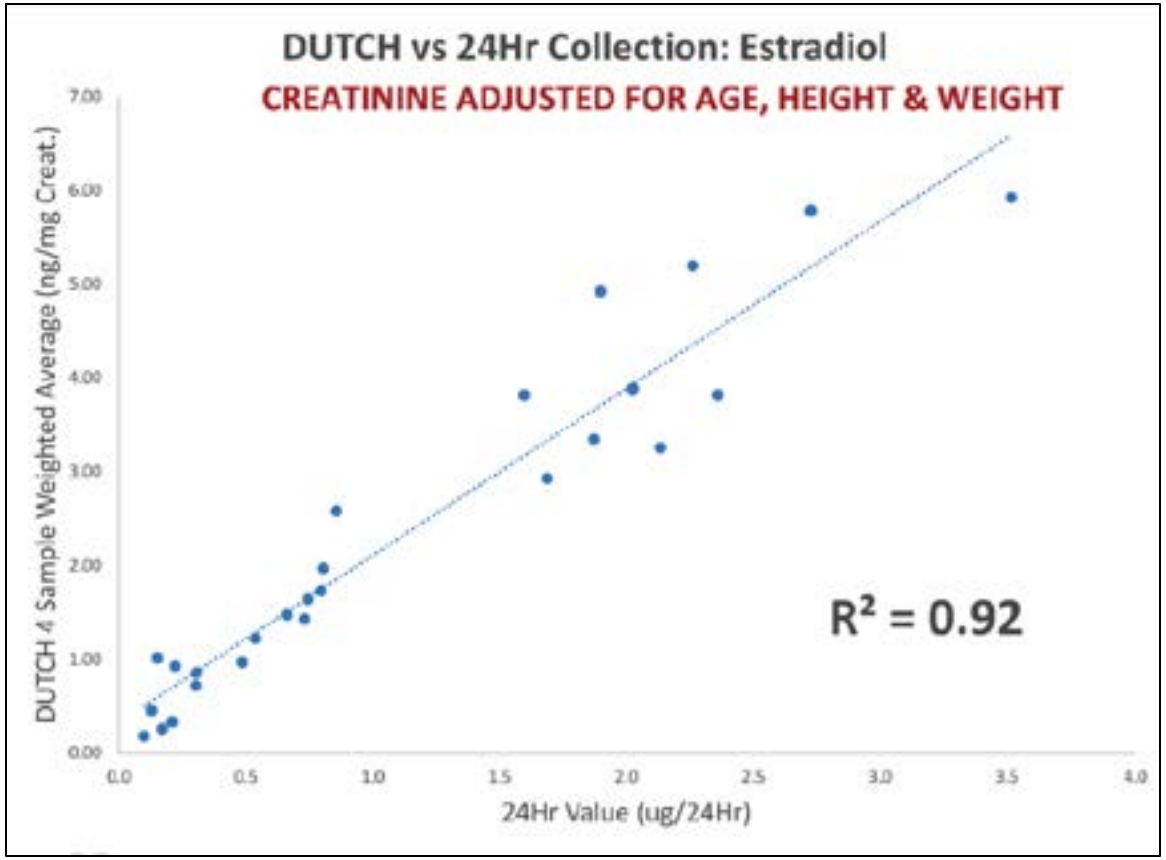


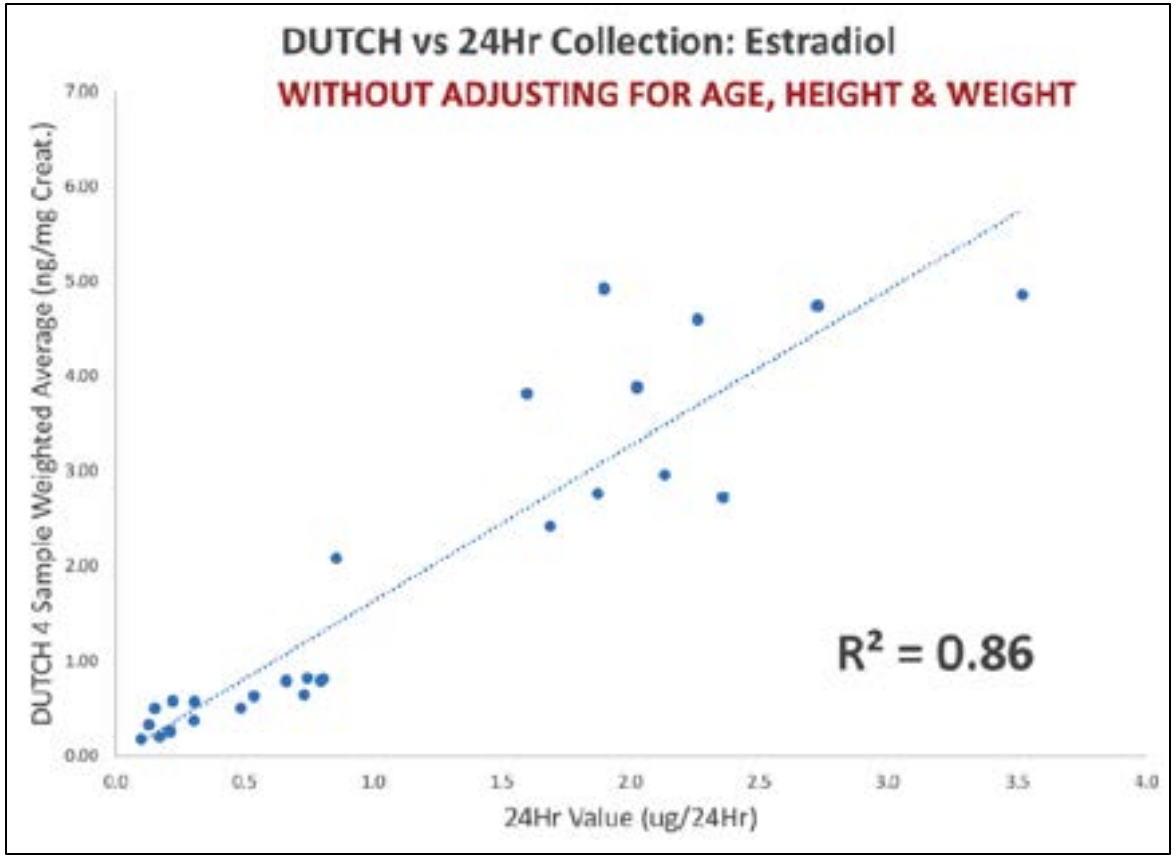
Estradiol (E2)

H = 4.2ng/mL
Creatinine = 2.0mg/mL
H = **2.1ng/mg** Creatinine



DUTCH Peer-Reviewed Published Validation vs Gold Standard Methods





DUTCH Peer-Reviewed Published Validation vs Gold Standard Methods

1. Newman M, Pratt SM, Curran DA, Stanczyk FZ. Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC–MS/MS). *BMC Chem*. 2019;13(1):1-12. doi:10.1186/s13065-019-0539-1

2. Newman M, Curran DA, Mayfield BP. Dried Urine and Salivary Profiling for Complete Assessment of Cortisol and Cortisol Metabolites. *J Clin Transl Endocrinol*. 2020;22:100243. doi:10.1016/j.jcte.2020.100243

3. Newman M, Curran DA. Reliability of a dried urine test for comprehensive assessment of urine hormones and metabolites. *BMC Chem*. 2021;15(1):18. doi:10.1186/s13065-021-00744-3

4. Newman MS, Curran DA, Mayfield BP, Saltiel D, Stanczyk FZ. Assessment of estrogen exposure from transdermal estradiol gel therapy with a dried urine assay. *Steroids*. 2022;184:109038. doi:10.1016/j.steroids.2022.109038

5. Newman MS, Mayfield BP, Saltiel D, Stanczyk FZ. Assessing estrogen exposure from transdermal estradiol patch therapy using a dried urine collection and a GC–MS/MS assay. *Steroids*. 2023;189:109149. doi:10.1016/j.steroids.2022.109149

6. Newman MS, Saltiel D, Smeaton J, Stanczyk FZ. Comparative estrogen exposure from compounded transdermal estradiol creams and Food and Drug Administration-approved transdermal estradiol gels and patches. *Menopause*. Published online October 18, 2023. doi:10.1097/GME.0000000000002266

7. Newman MS, Smeaton J. Comprehensive assessment of cortisol and cortisol metabolites provides insight into the complex relationship between HPA axis function and BMI. *Endocr Metab Sci*. 2023;13:100147. doi:10.1016/j.endmts.2023.100147

8. Newman M, Mayfield BP, Saltiel D, Stanczyk FZ. Monitoring Estradiol Patch Therapy with a Validated Dried Urine Assay. Poster Presented at: The 2021 Annual Meeting of The North American Menopause Society; September 22-25, 2021; Washington, D.C. doi:10.1097/GME.0000000000001890

9. Newman M, Mayfield BP, Saltiel D, Stanczyk FZ. Monitoring Transdermal Estradiol Gel Therapy with a Validated Dried Urine Assay. Poster Presented at: The 2021 Annual Meeting of The North American Menopause Society; September 22-25, 2021; Washington, D.C. doi:10.1097/GME.0000000000001890

10. Newman M, Mayfield BP, Saltiel D, Stanczyk FZ. Comparing Urinary Estrogen Profiles of Women on Transdermal Estradiol Patches, Gels, or Creams. Poster Presented at: The 2021 Annual Meeting of The North American Menopause Society; September 22-25, 2021; Washington, D.C. doi:10.1097/GME.0000000000001890

11. Newman M, Saltiel D, Curran DA. Approximating Systemic Estrogen Exposure from Vaginal Estrogen Cream Therapy. Poster Presented at: The 2022 Annual Meeting of The North American Menopause Society; October 12-15, 2022; Atlanta, GA. doi:10.1097/GME.0000000000002112

12. Newman M, Saltiel D, Mayfield BP, Stanczyk FZ. The Impact of 3,3'-Diindolylmethane on the Estrogen Profile of Postmenopausal Women Being Treated with a Transdermal Estradiol Patch. Poster Presented at: The 2022 Annual Meeting of The North American Menopause Society; October 12-15, 2022; Atlanta, GA. doi:10.1097/GME.0000000000002112

13. Newman M, Saltiel D, Mayfield BP, Stanczyk FZ. Sensitivity, Specificity, and Predictive Value of Urinary Androgen Metabolites for the Diagnosis of Polycystic Ovary Syndrome. Poster presented at: The 78th Scientific Congress of the American Society for Reproductive Medicine; October 22-26, 2022; Anaheim, CA. *Fertil Steril*. 2022;118(4):e209. doi:10.1016/j.fertnstert.2022.08.594

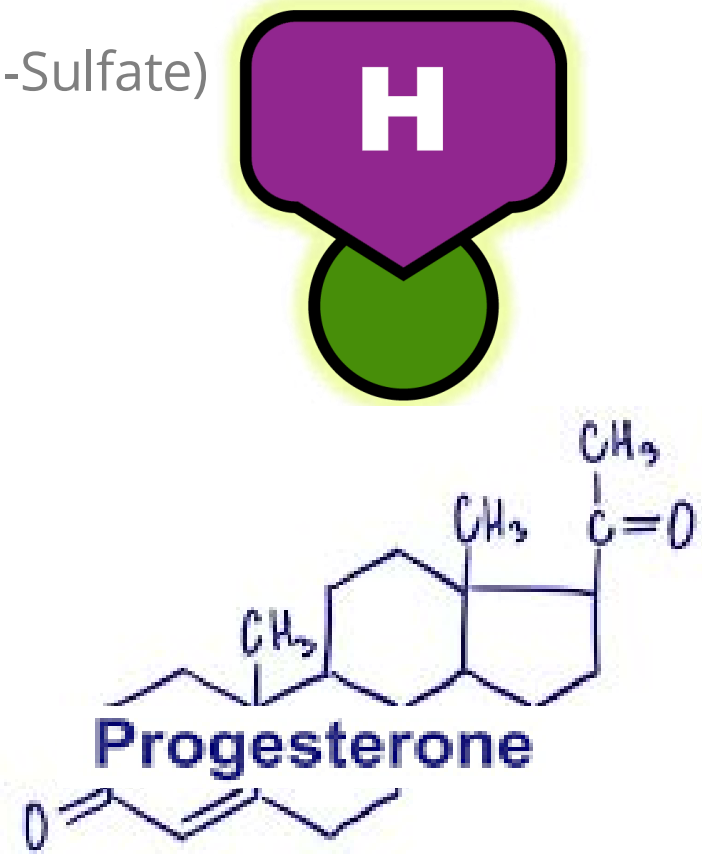
14. Newman M, Saltiel D, Mayfield BP, Stanczyk FZ. Urinary Estrogen and Progesterone Metabolite Patterns in Ovulatory and Anovulatory Women. Poster presented at: The 78th Scientific Congress of the American Society for Reproductive Medicine; October 22-26, 2022; Anaheim, CA. *Fertil Steril*. 2022;118(4):e211. doi:10.1016/j.fertnstert.2022.08.599

15. Newman M, Saltiel D, Mayfield BP, Stanczyk FZ. Comparison of Cyclic Urinary Estrogen and Progesterone Metabolite Patterns Between Women Reporting Menses and Women Reporting No Menses. Poster presented at: The 78th Scientific Congress of the American Society for Reproductive Medicine; October 22-26, 2022; Anaheim, CA. *Fertil Steril*. 2022;118(4):e211-e212. doi:10.1016/j.fertnstert.2022.08.600

dutch®

How Can Contamination Impact DUTCH

- Topical/Vaginal Hormones – a problem solved with **clever lab methodology**
  - Not an issue for progesterone
  - Exception – Vaginal Premarin (Estrone-Sulfate)



How Can Contamination Impact DUTCH

- Topical/Vaginal Hormones – a problem solved with **clever lab methodology**
  - Not an issue for progesterone
  - Exception – Vaginal Premarin

- Cortisol (hydrocortisone) creams
- Typical dose may be 25mg = 25,000,000ng!

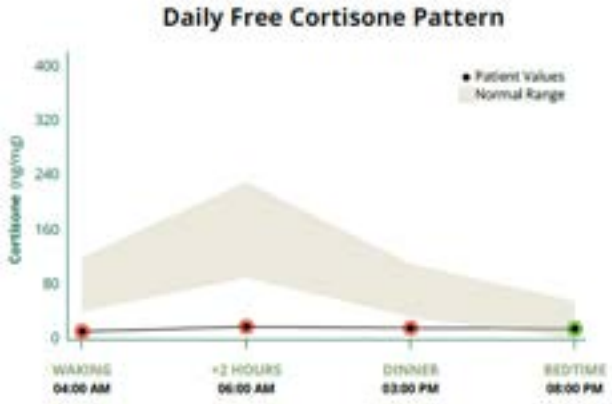
Cortisol Abnormalities – Real?

What can we learn from page 1?

- Weird cortisol
- Low DHEA production

What can we learn from page 3?

- Cortisone is NOT high
- Hydrocortisone contamination!



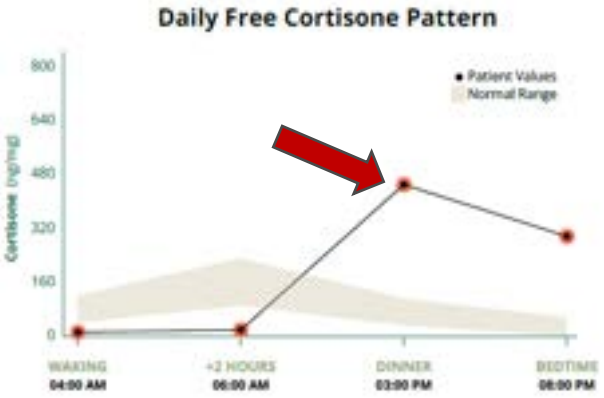
Cortisol Abnormalities – Real?

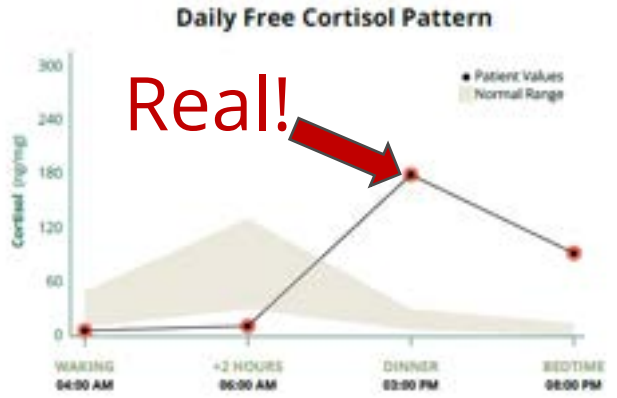
What can we learn from page 1?

- Weird cortisol
- NOT low cortisol production
- But does “production” mean “produced”?

What can we learn from page 3?

- Cortisone is ALSO high
- NOT contamination!
- Oral cortisol with suppression?
- Or Prednisone taken after dinner?

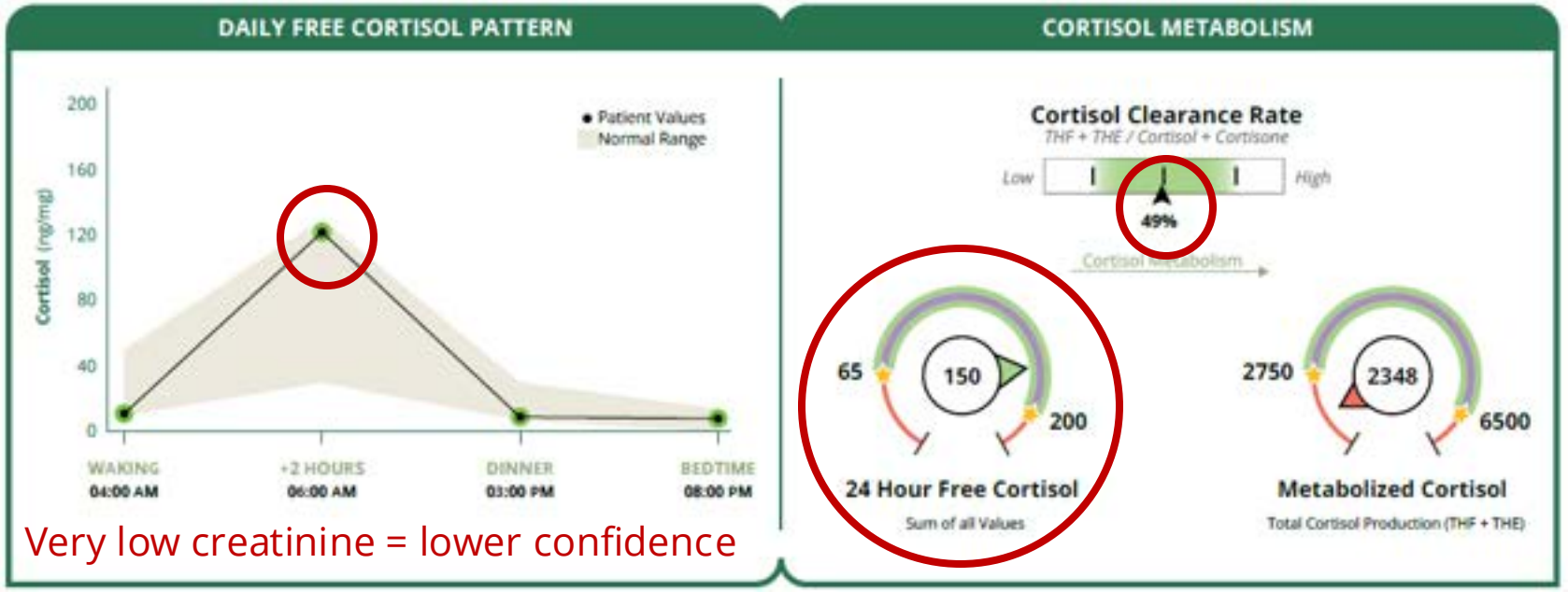




How Can a DUTCH Test Lie to You?

- Oral supplementation (beware melatonin, DHEA, etc.)
- Timing, collection issues
- Contamination (reduced with DUTCH methods)
- **Creatinine anomalies**
- Phase 2 metabolism anomalies (more on this later today)

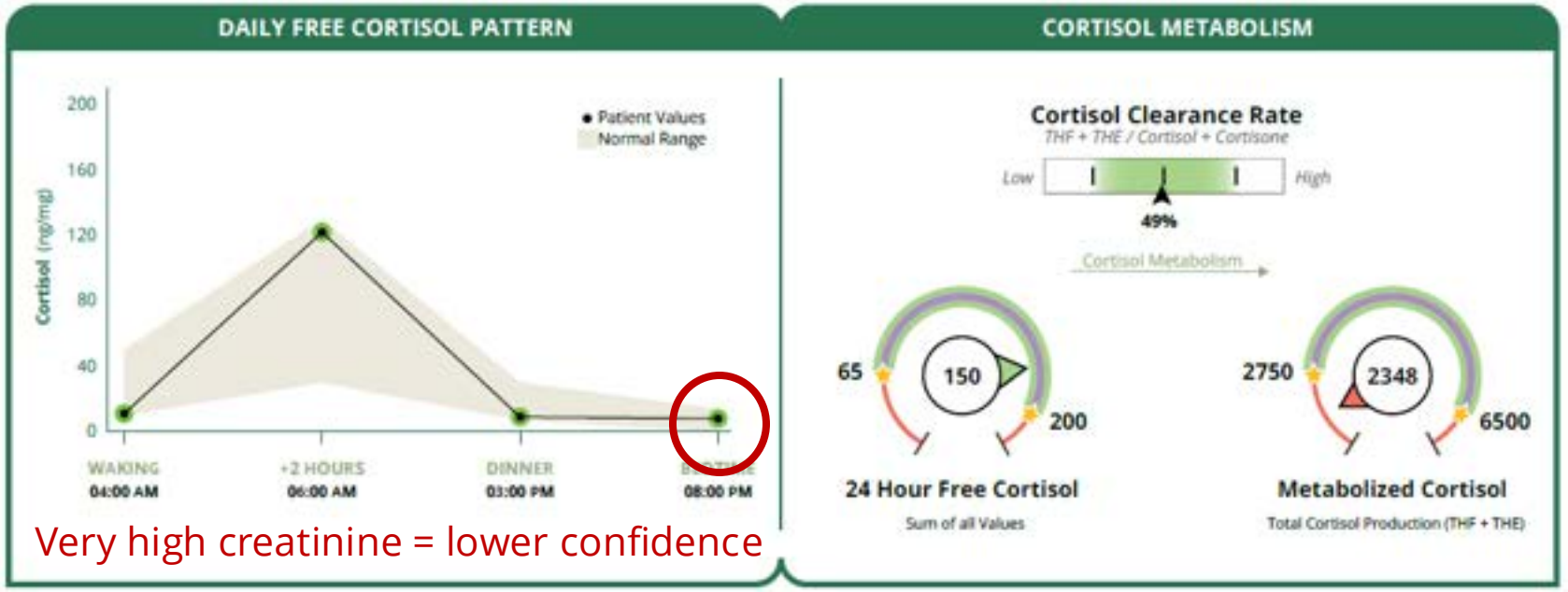
Cortisol Abnormalities – Real?



Very low creatinine = lower confidence

TEST		RESULT	UNITS	NORMAL RANGE
Creatinine (Urine)				
Creatinine (U1) - Waking	Within range	1.07	mg/ml	0.2 - 2
Creatinine (U2) - +2 Hours	Below range	0.10	mg/ml	0.2 - 2
Creatinine (U3) - Dinner	Within range	0.92	mg/ml	0.2 - 2
Creatinine (U4) - Bedtime	Above range	2.22	mg/ml	0.2 - 2

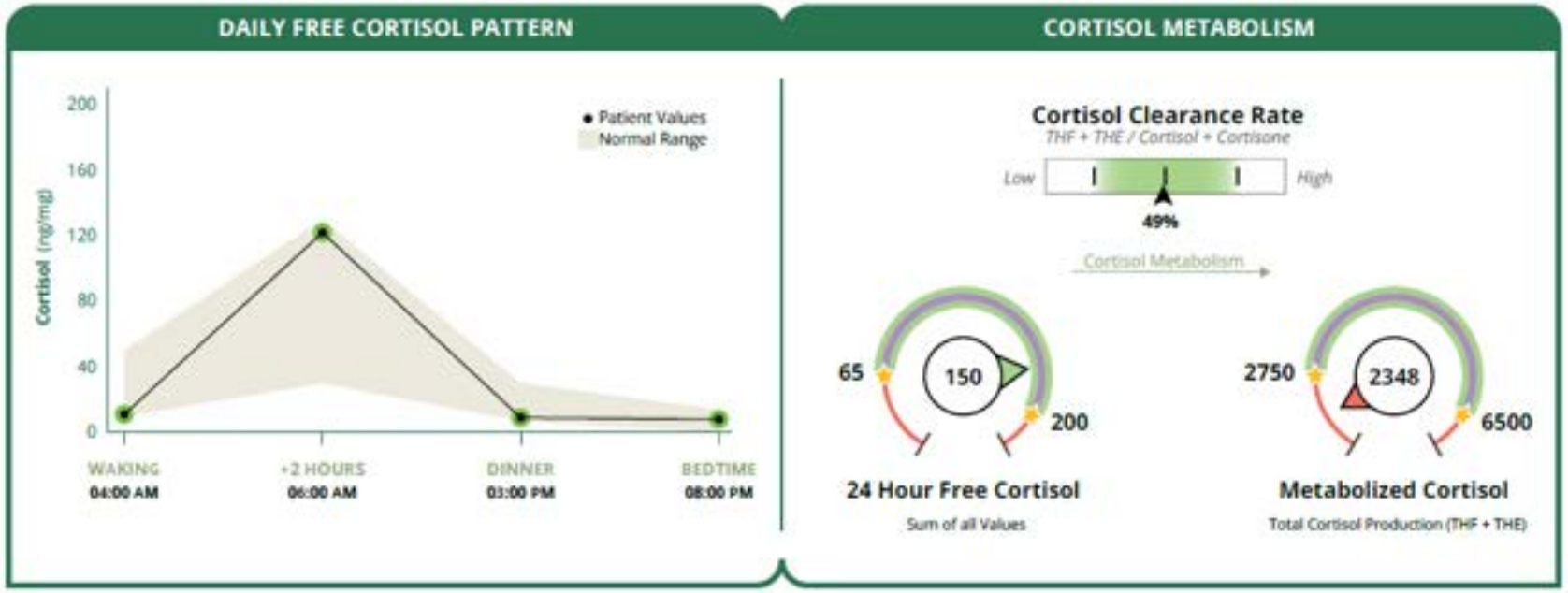
Cortisol Abnormalities – Real?



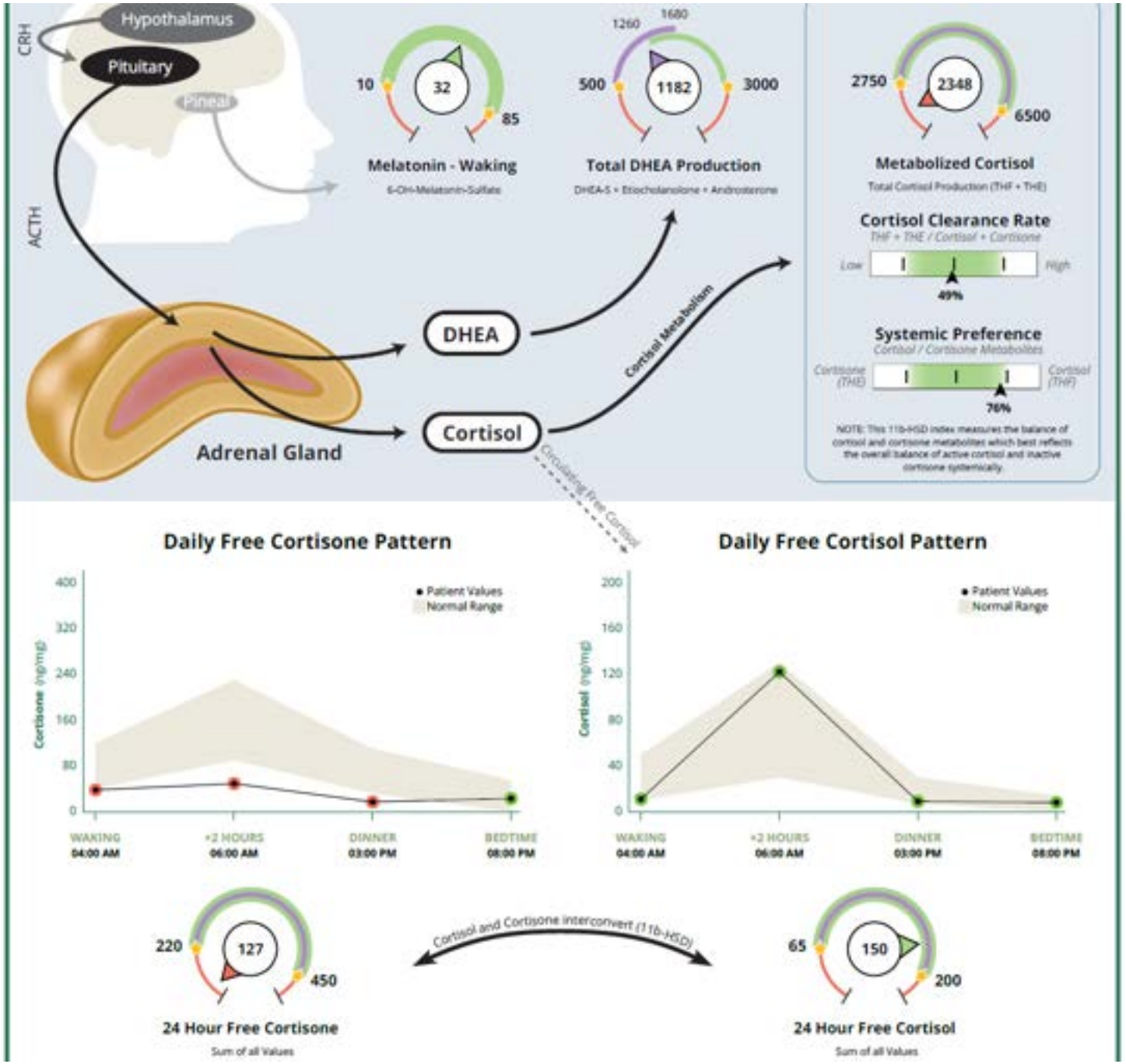
Very high creatinine = lower confidence

TEST		RESULT	UNITS	NORMAL RANGE
Creatinine (Urine)				
Creatinine (U1) - Waking	Within range	1.07	mg/ml	0.2 - 2
Creatinine (U2) - +2 Hours	Below range	0.10	mg/ml	0.2 - 2
Creatinine (U3) - Dinner	Within range	0.92	mg/ml	0.2 - 2
Creatinine (U4) - Bedtime	Above range	2.22	mg/ml	0.2 - 2

Cortisol Abnormalities – Real?

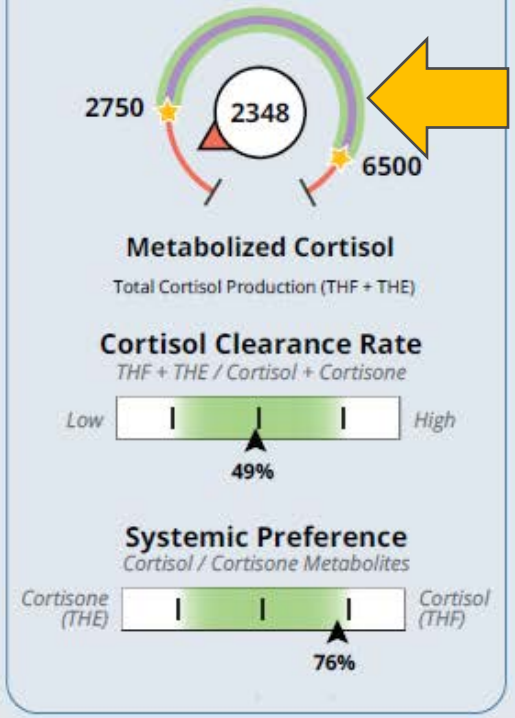


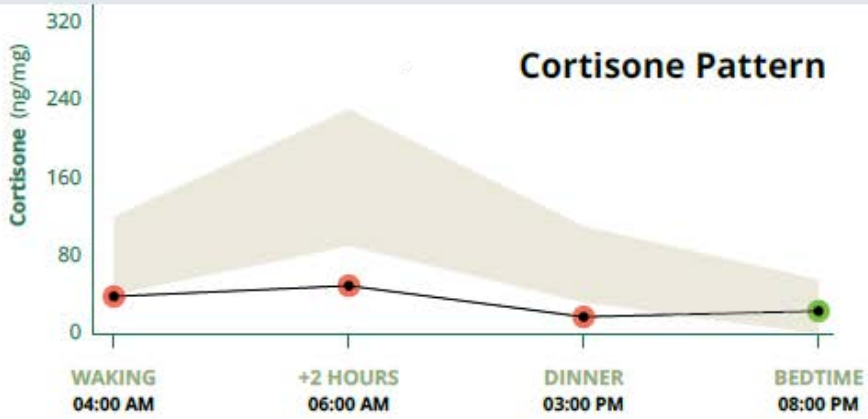
TEST		RESULT	UNITS	NORMAL RANGE
Creatinine (Urine)				
Creatinine (U1) - Waking	Within range	1.07	mg/ml	0.2 - 2
Creatinine (U2) - +2 Hours	Below range	0.10	mg/ml	0.2 - 2
Creatinine (U3) - Dinner	Within range	0.92	mg/ml	0.2 - 2
Creatinine (U4) - Bedtime	Above range	2.22	mg/ml	0.2 - 2

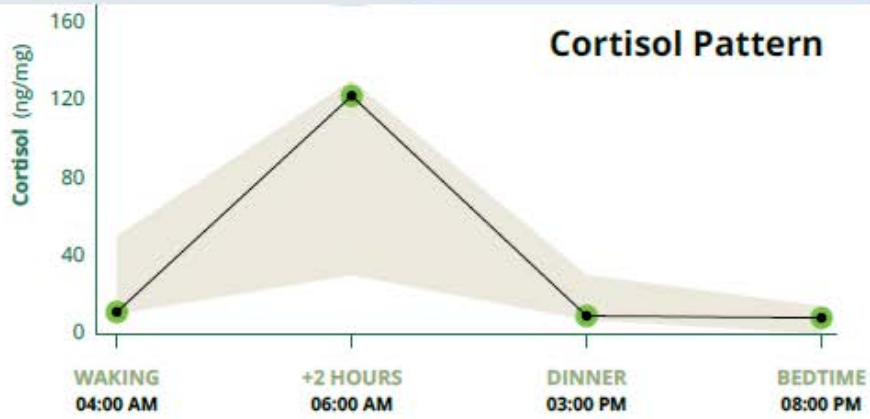

Hypothalamus
CRH
Pituitary
Pineal
ACTH
Adrenal Gland
DHEA
Cortisol
Cortisol Metabolism
Circulating Free Cortisol
10
32
85
Melatonin - Waking
6-OH-Melatonin-Sulfate
1260
1680
500
1182
3000
Total DHEA Production
DHEA-S + Etiocholanolone + Androsterone
2750
2348
6500
Metabolized Cortisol
Total Cortisol Production (THF + THE)
Cortisol Clearance Rate
THF + THE / Cortisol + Cortisone
Low
High
49%
Systemic Preference
Cortisol / Cortisone Metabolites
Cortisone (THE)
Cortisol (THF)
76%
NOTE: This 11b-HSD index measures the balance of cortisol and cortisone metabolites which best reflects the overall balance of active cortisol and inactive cortisone systemically.
Daily Free Cortisone Pattern
Patient Values
Normal Range
Cortisone (ng/mg)
400
320
240
160
80
0
WAKING
04:00 AM
+2 HOURS
06:00 AM
DINNER
03:00 PM
BEDTIME
08:00 PM
Daily Free Cortisol Pattern
Patient Values
Normal Range
Cortisol (ng/mg)
200
160
120
80
40
0
WAKING
04:00 AM
+2 HOURS
06:00 AM
DINNER
03:00 PM
BEDTIME
08:00 PM
220
127
450
24 Hour Free Cortisone
Sum of all Values
Cortisol and Cortisone interconvert (11b-HSD)
65
150
200
24 Hour Free Cortisol
Sum of all Values

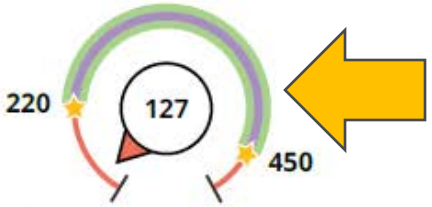
Interpretation Options???

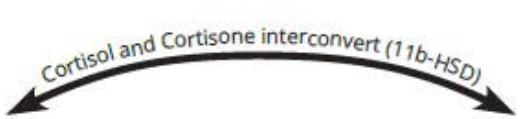
1. Free Cortisol and Metabolized Cortisol are not aligned
2. Free Cortisol (active) and Cortisone (inactive) are not aligned



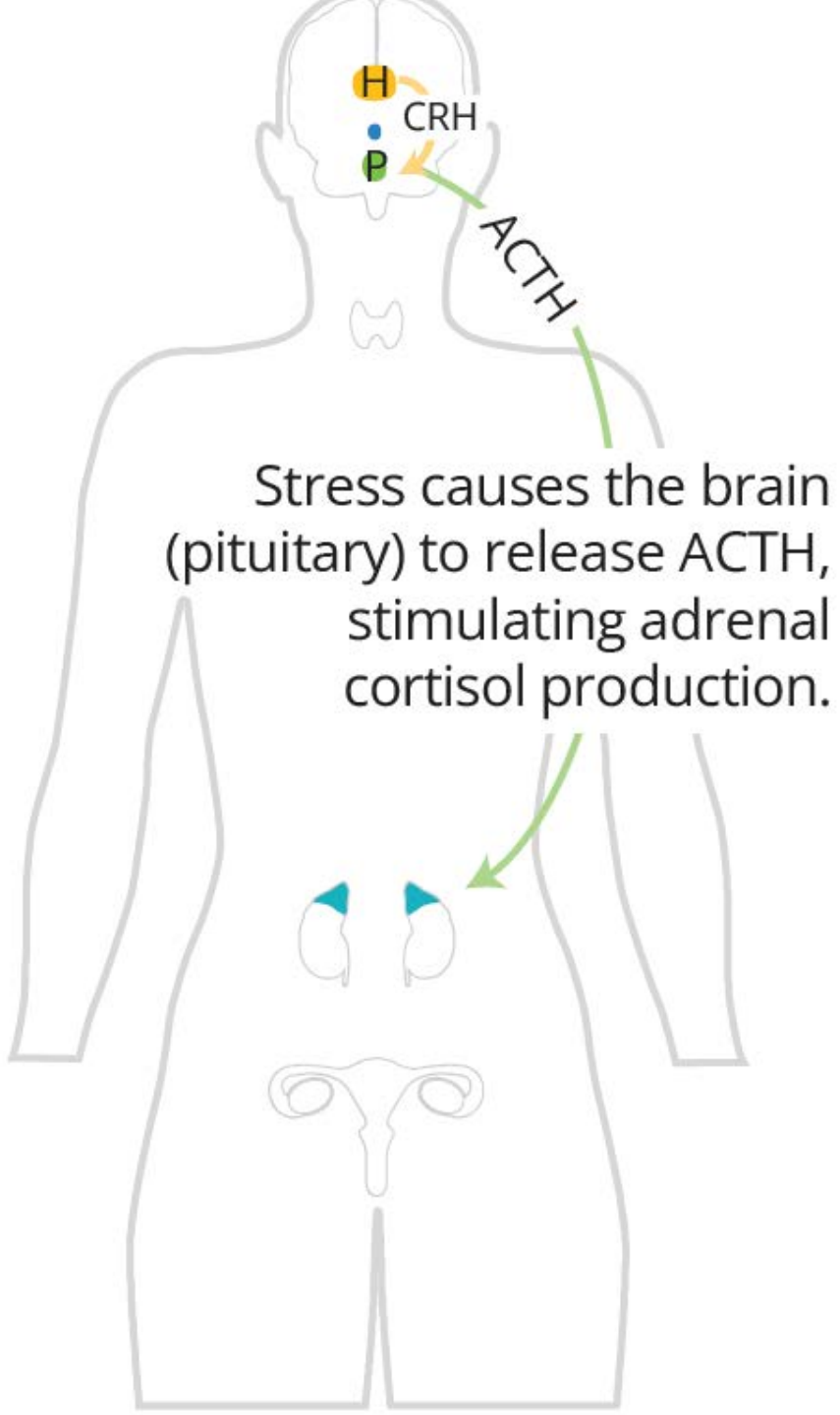


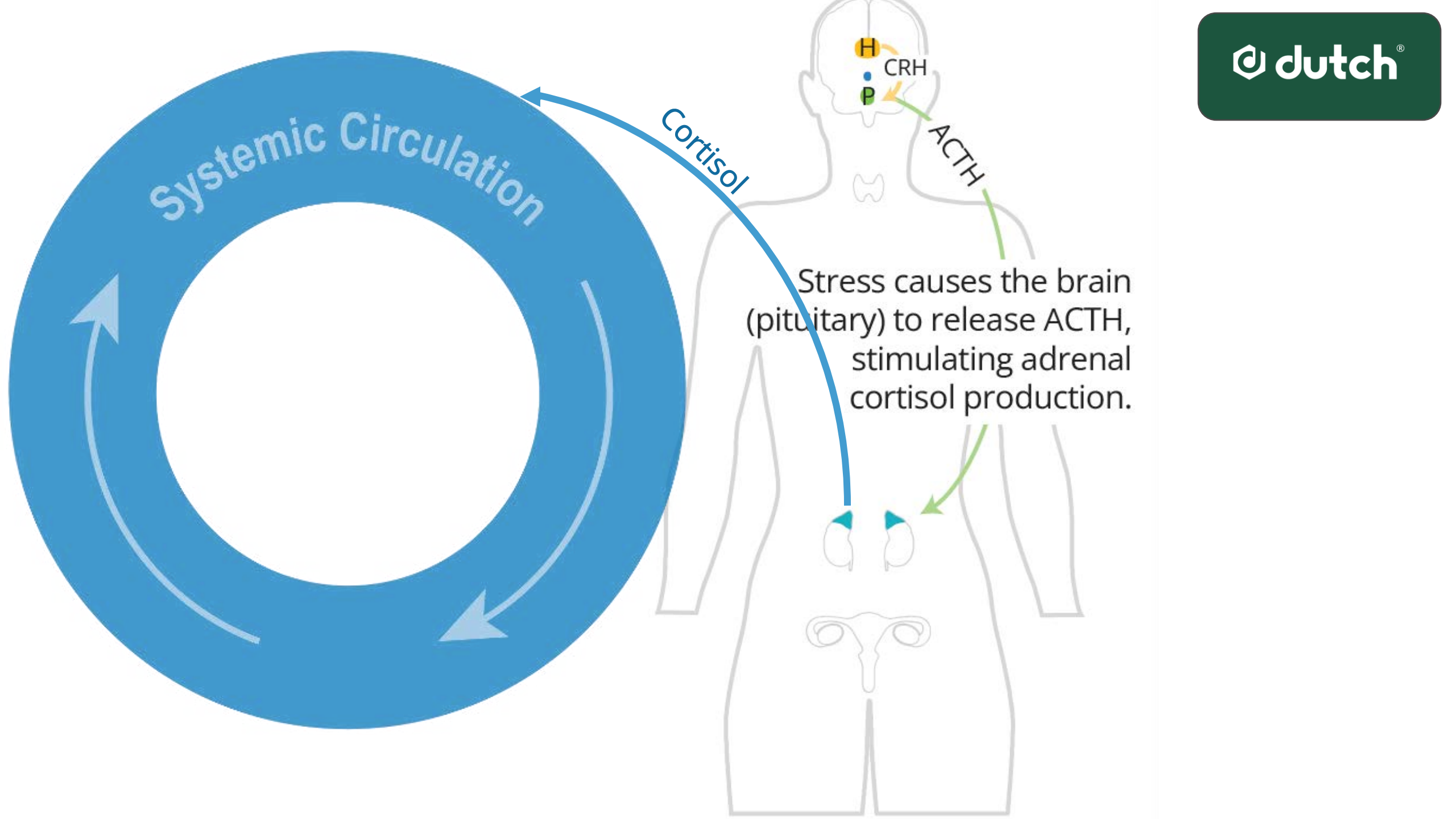






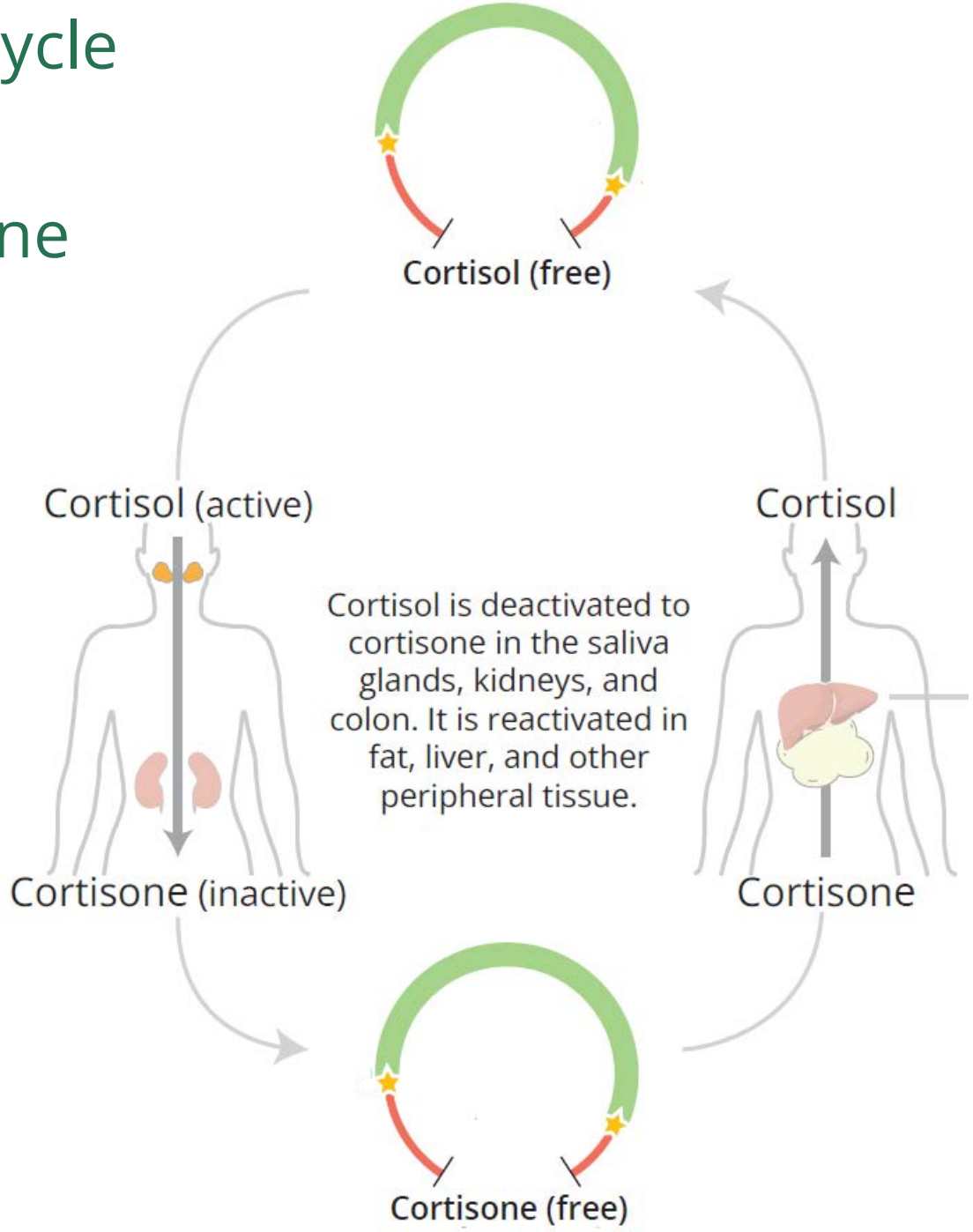
Let’s Talk About It!




Systemic Circulation
Cortisol
H
CRH
P
ACTH
Stress causes the brain (pituitary) to release ACTH, stimulating adrenal cortisol production.

Cortisol Metabolism Cycle

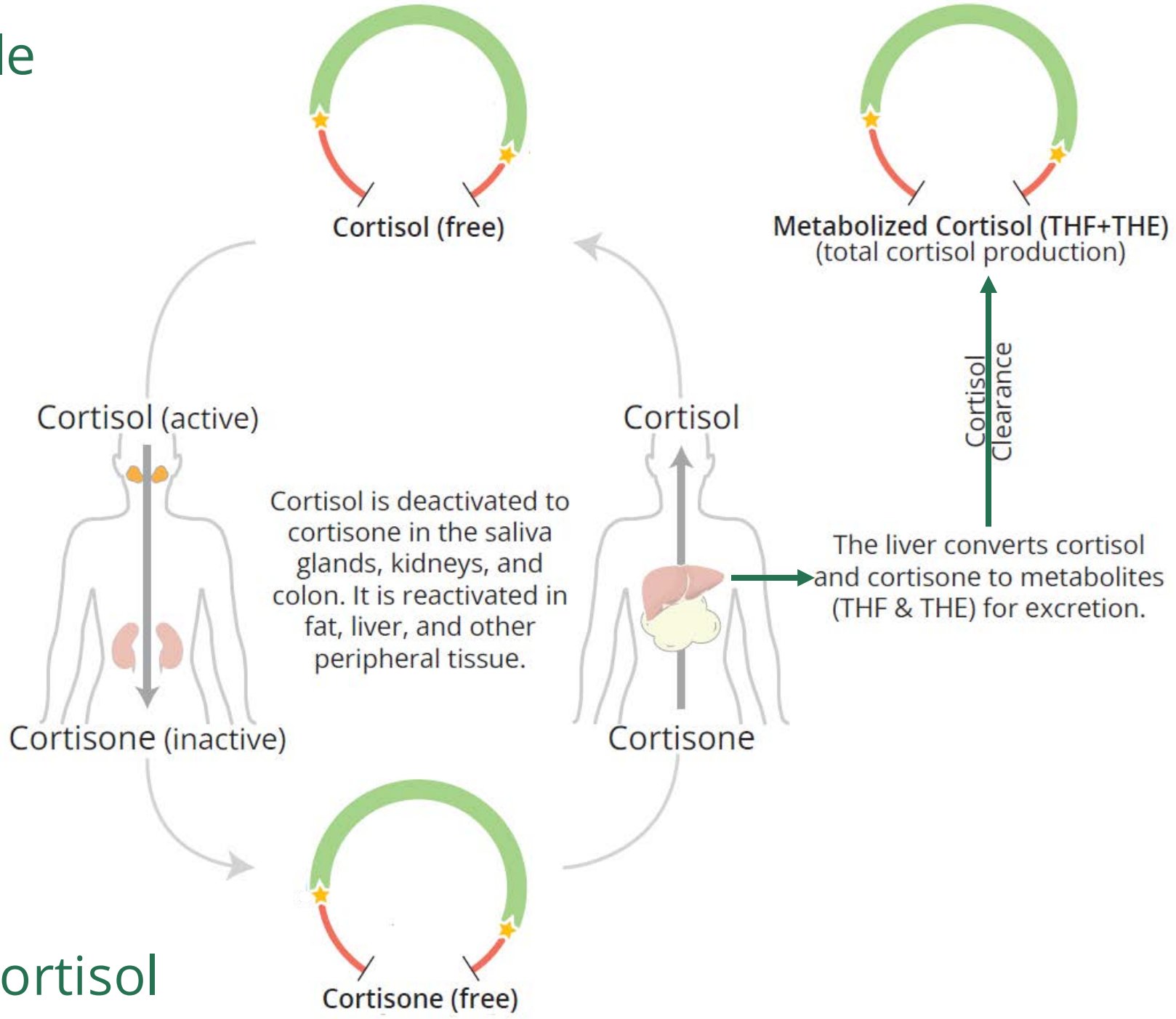
Cortisol ←→ Cortisone



Cortisol Metabolism Cycle Termination

Cortisol → THF

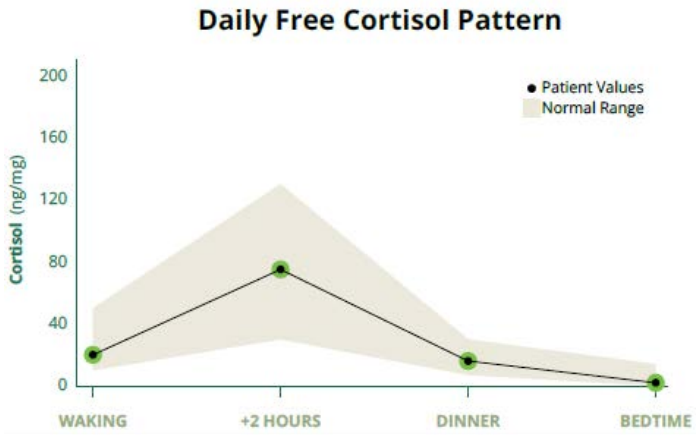
Cortisone → THE

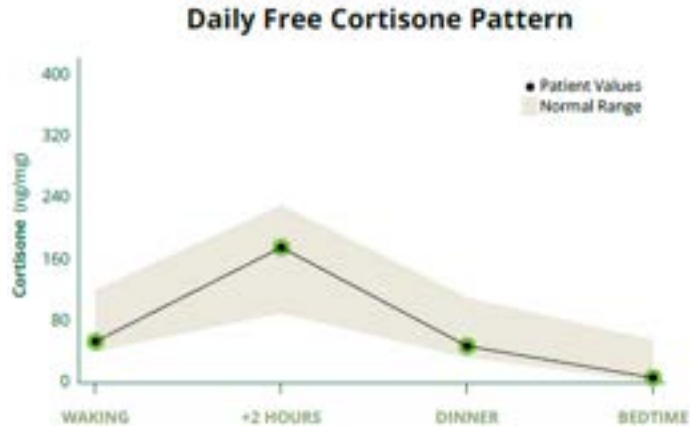


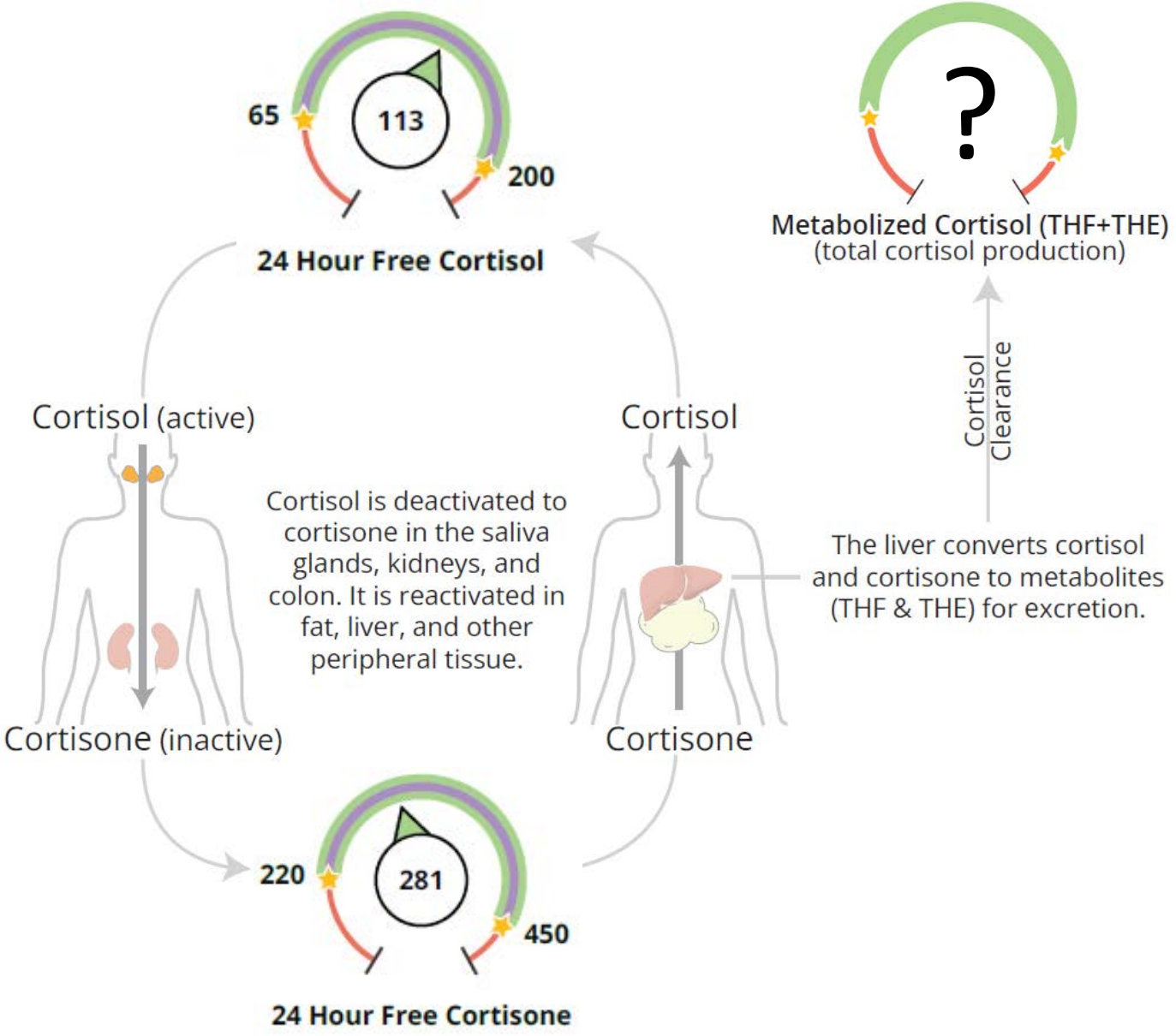
THF = Tetrahydrocortisol

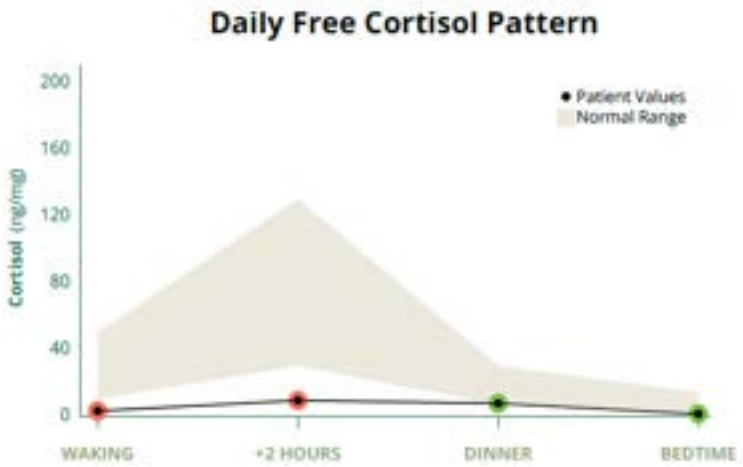
THE = Tetrahydrocortisone

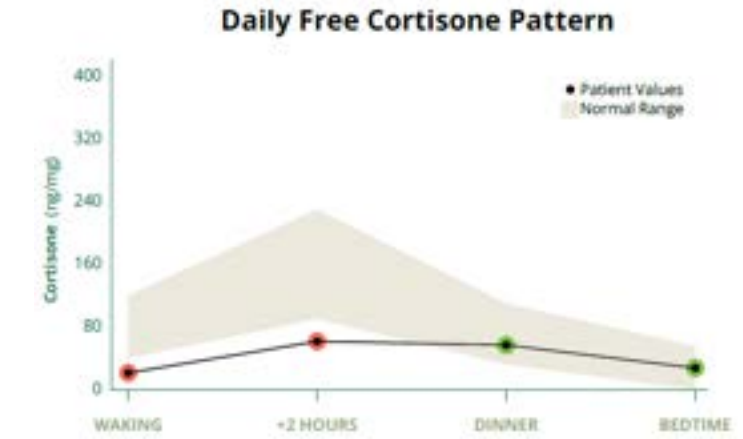
THF + THE = Metabolized Cortisol

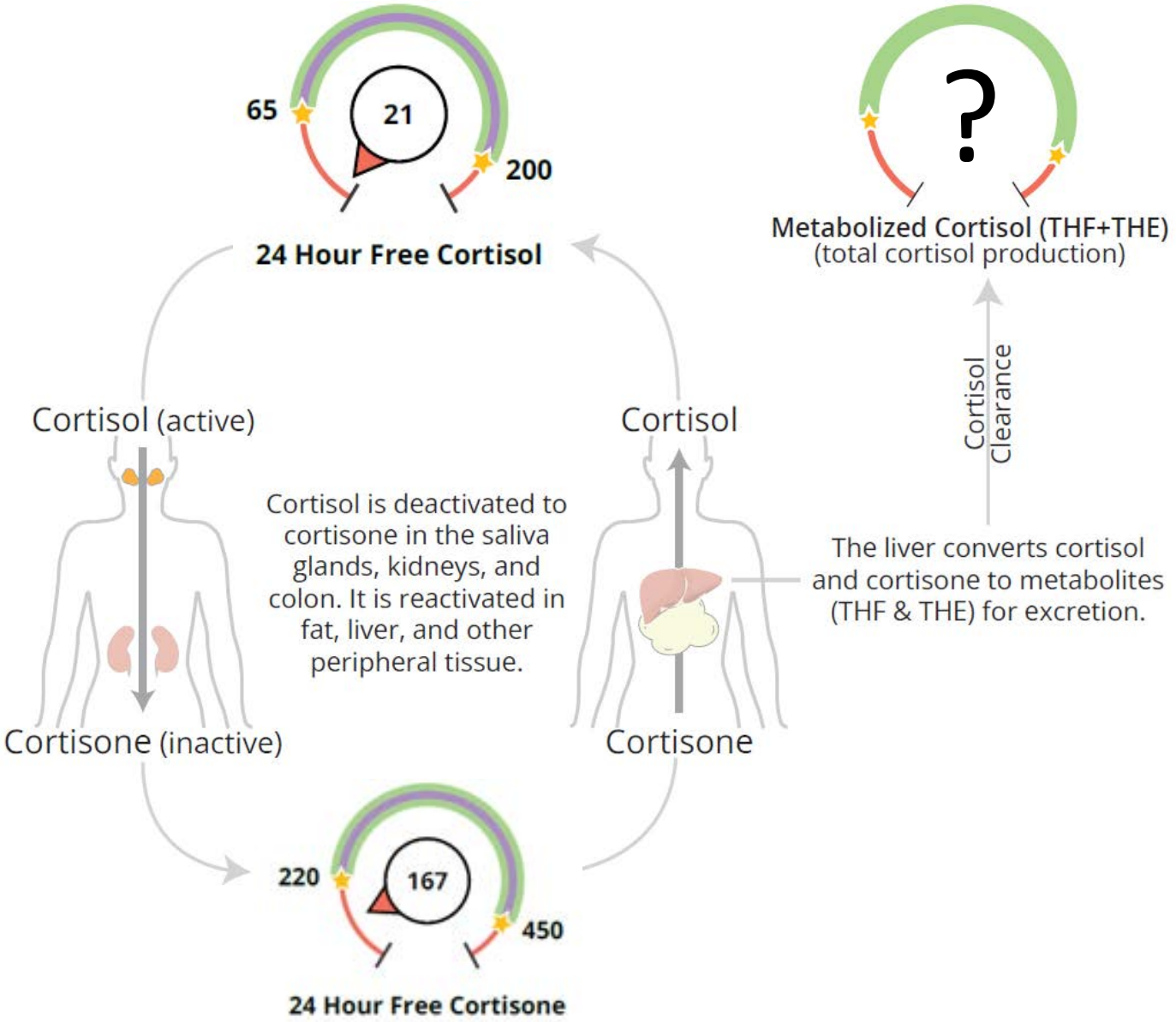


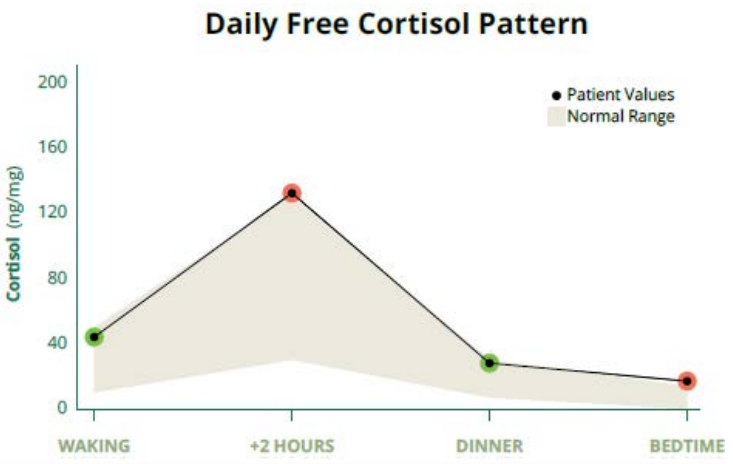


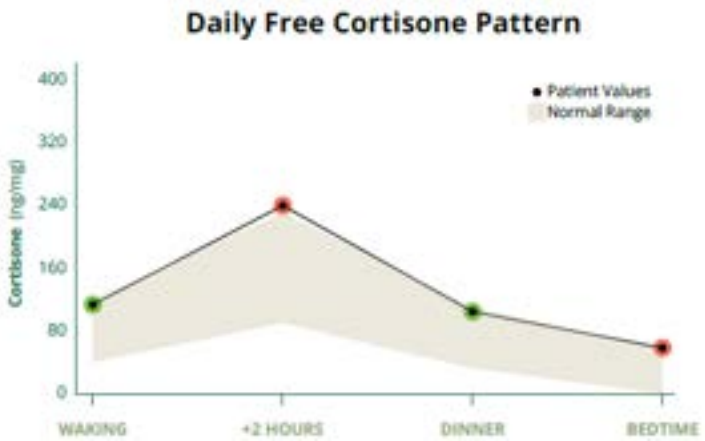


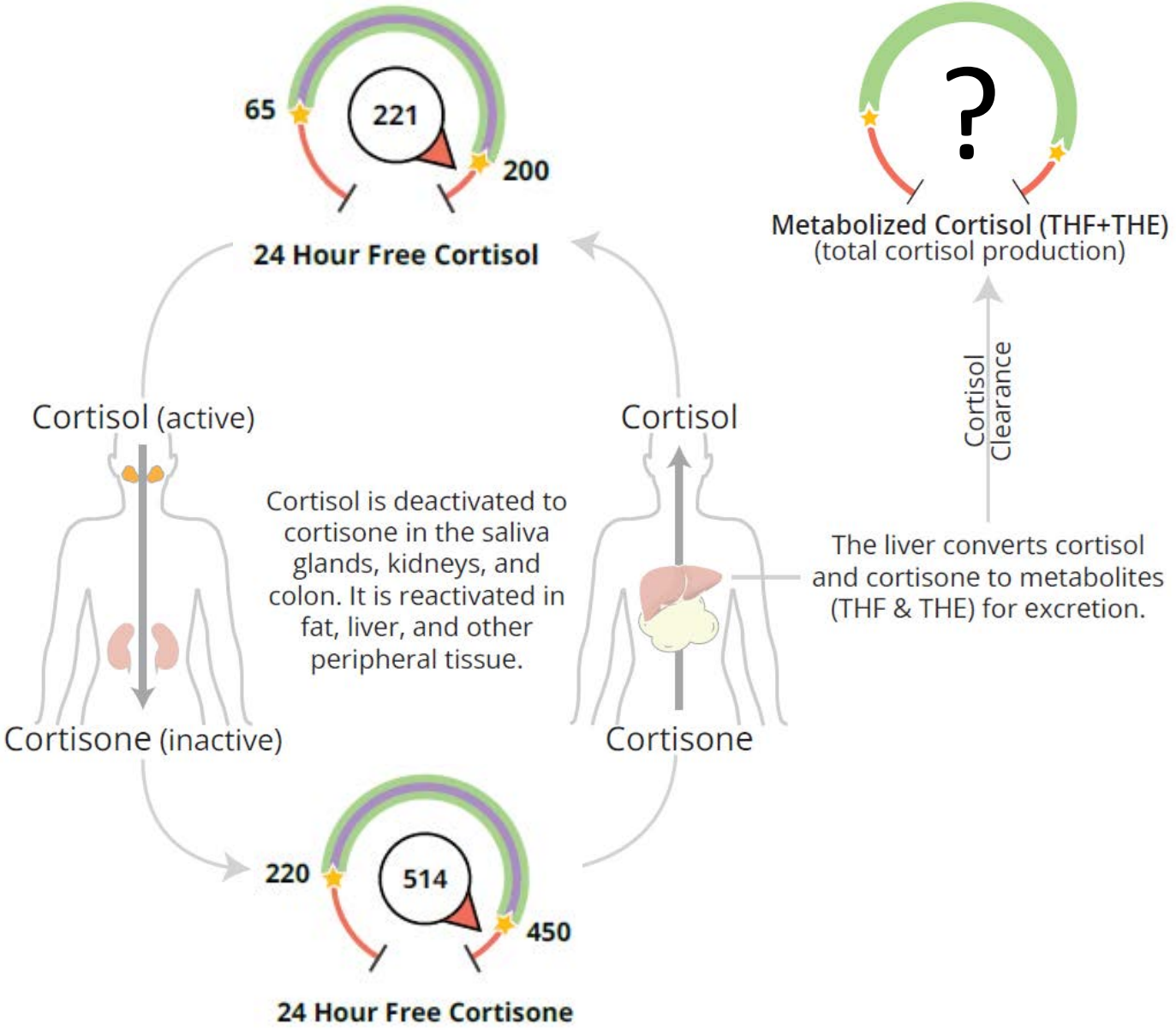




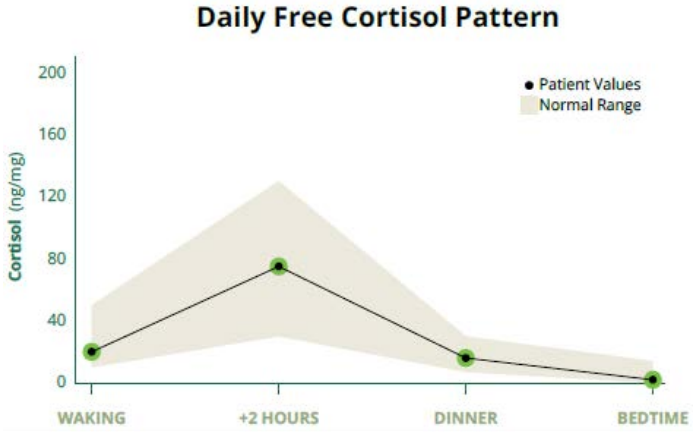


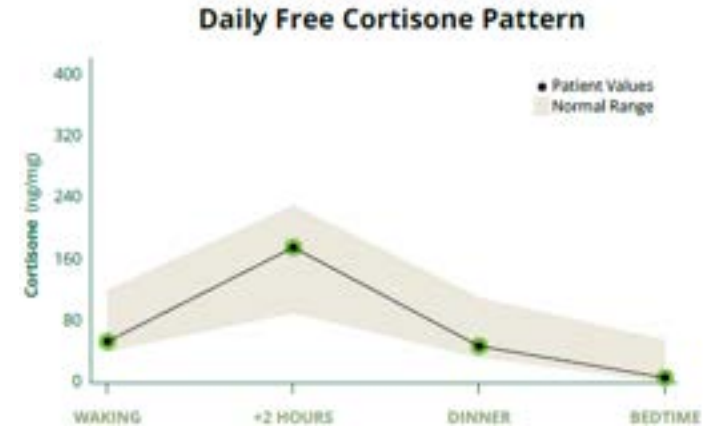


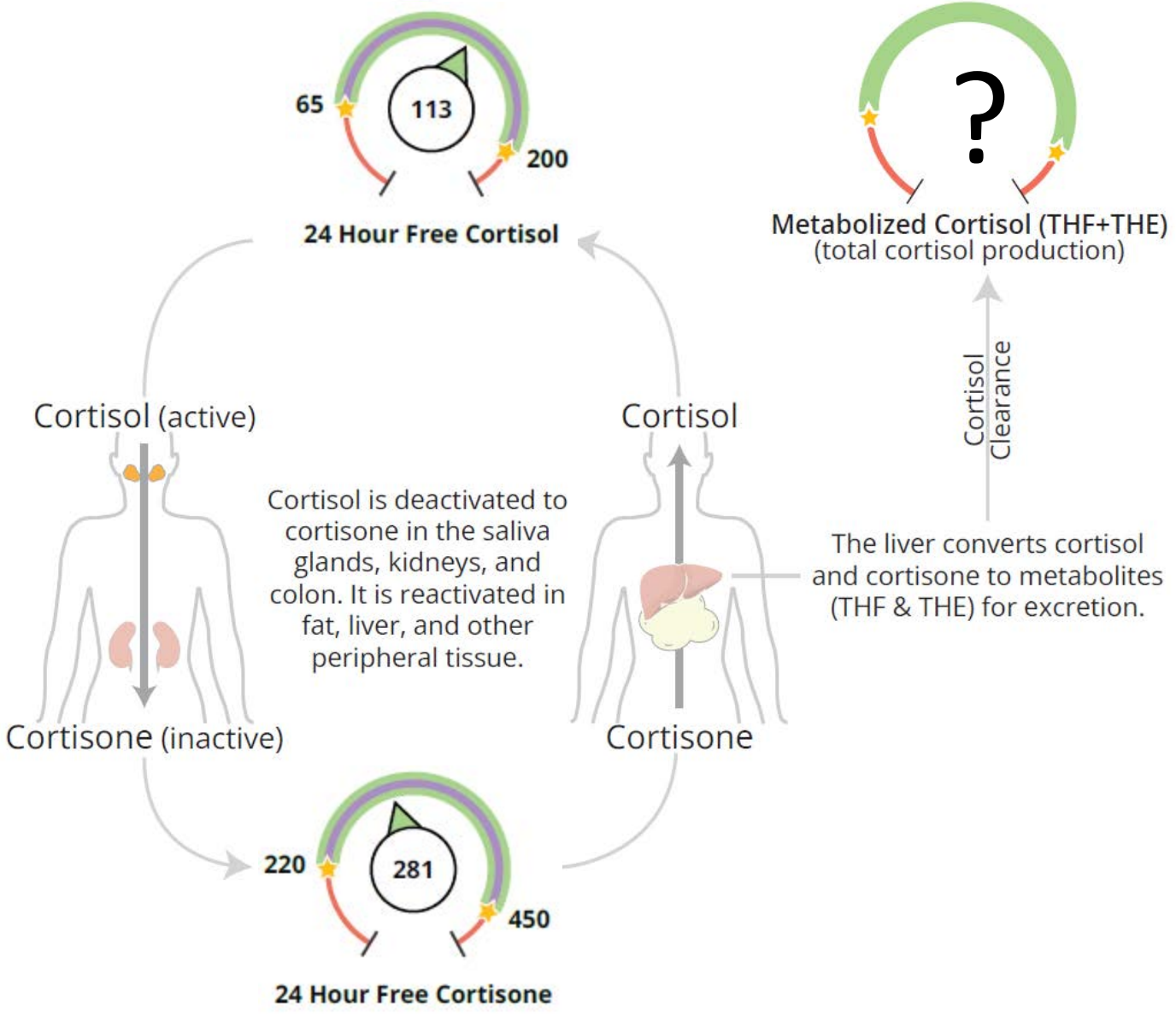


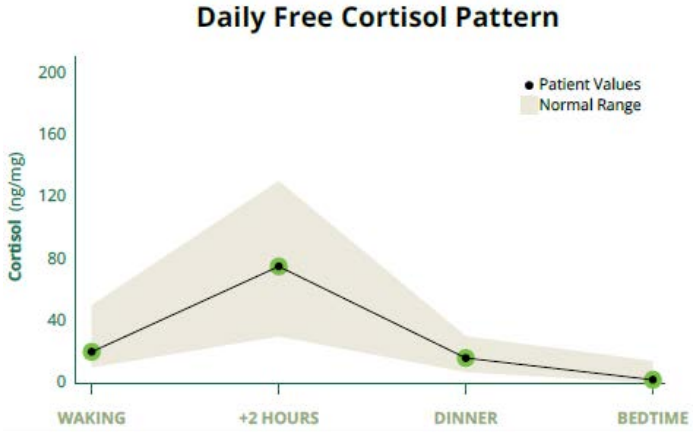


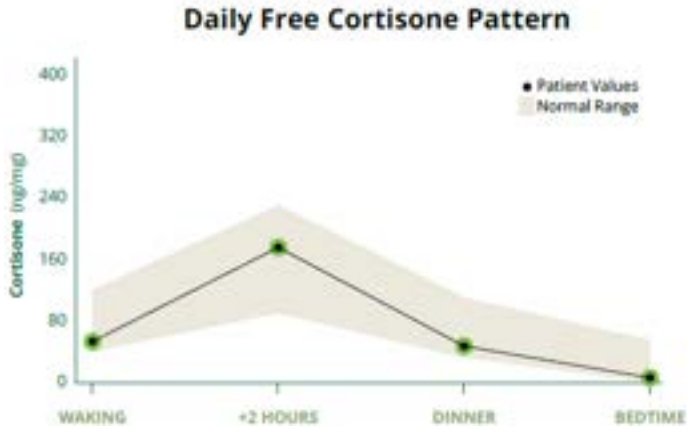
When Free and Metabolized Cortisol Don't Match

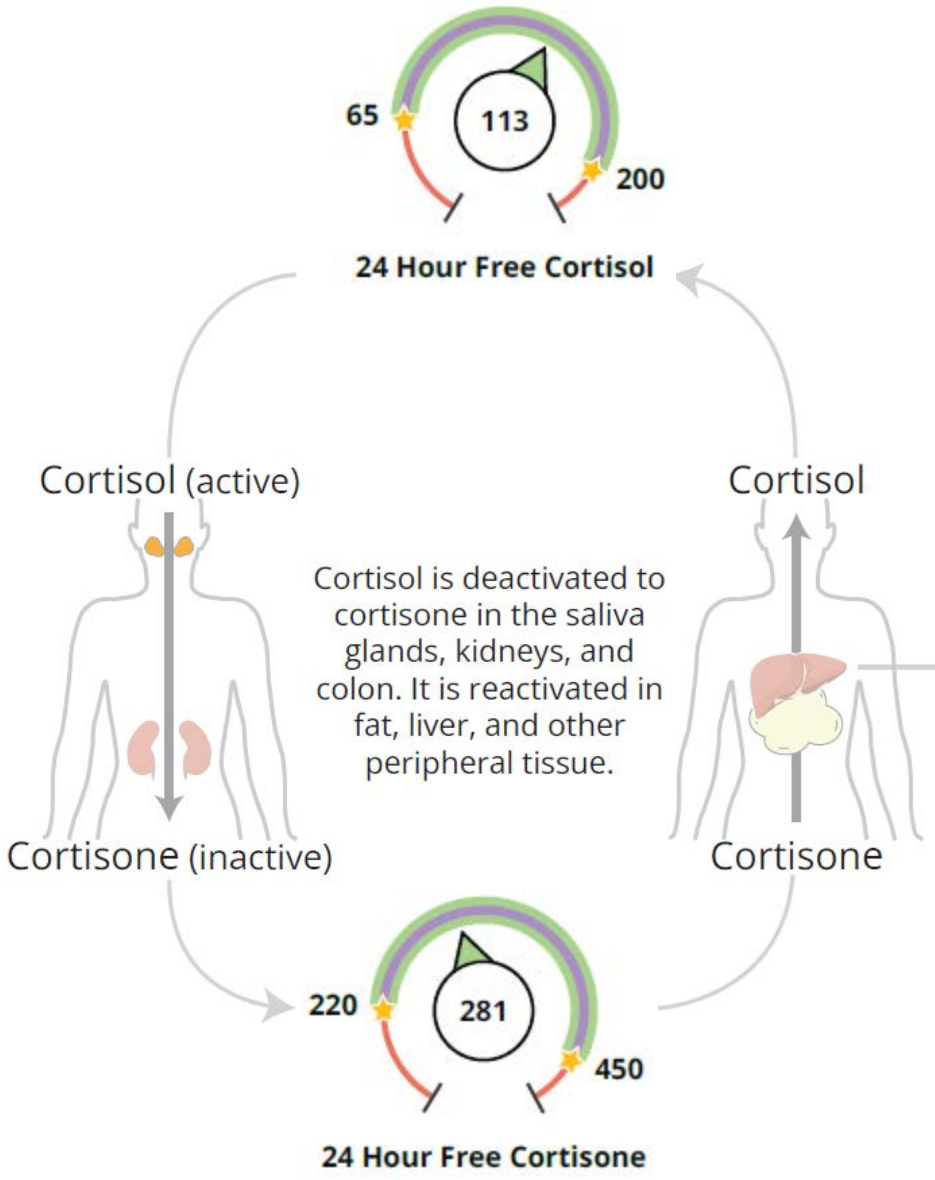


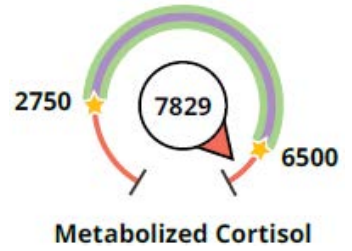




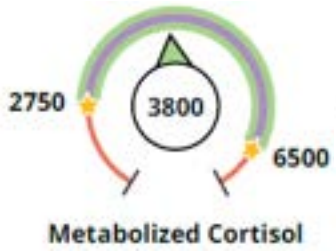


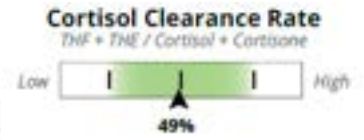


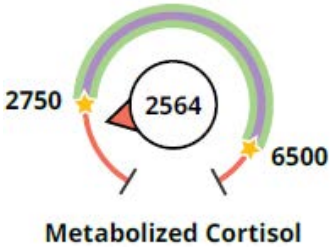




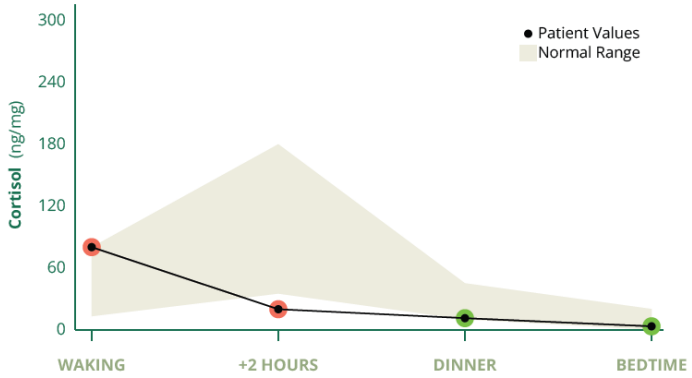
?



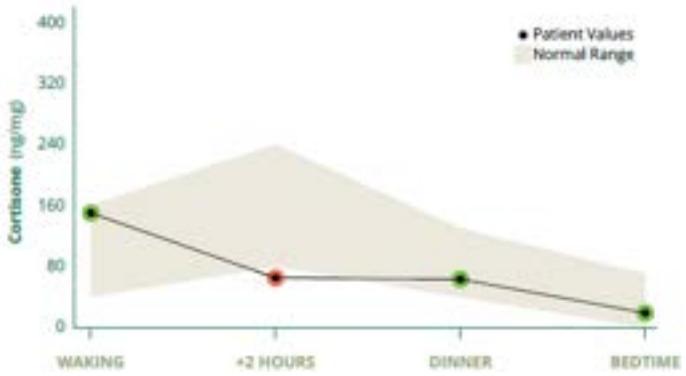


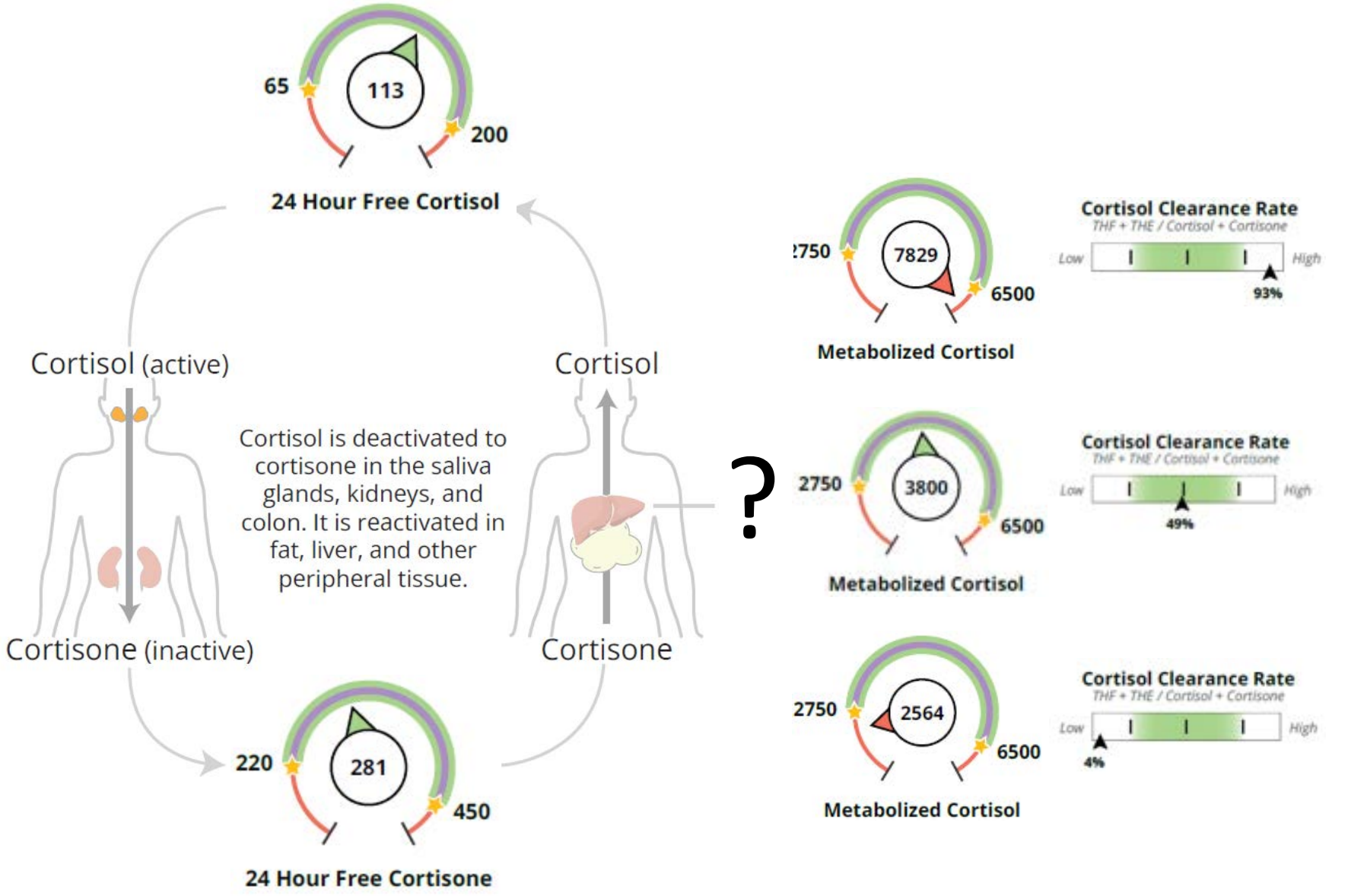


Daily Free Cortisol Pattern

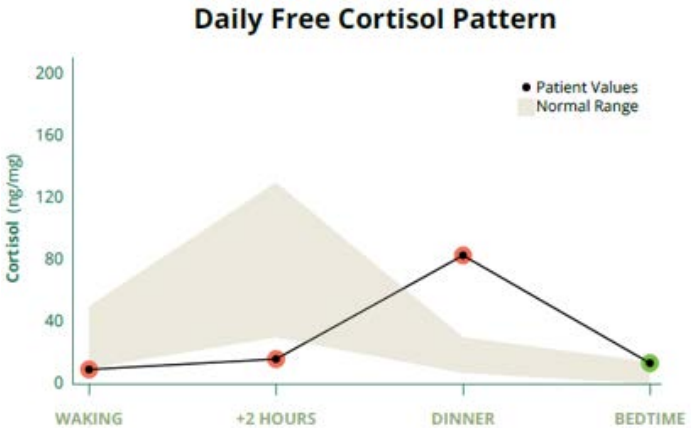


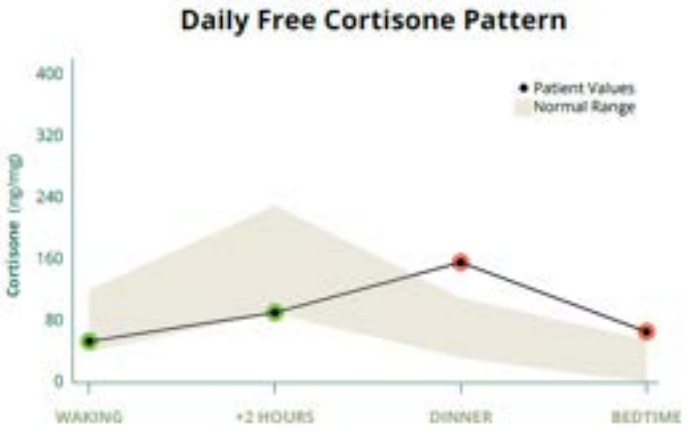
Daily Free Cortisone Pattern

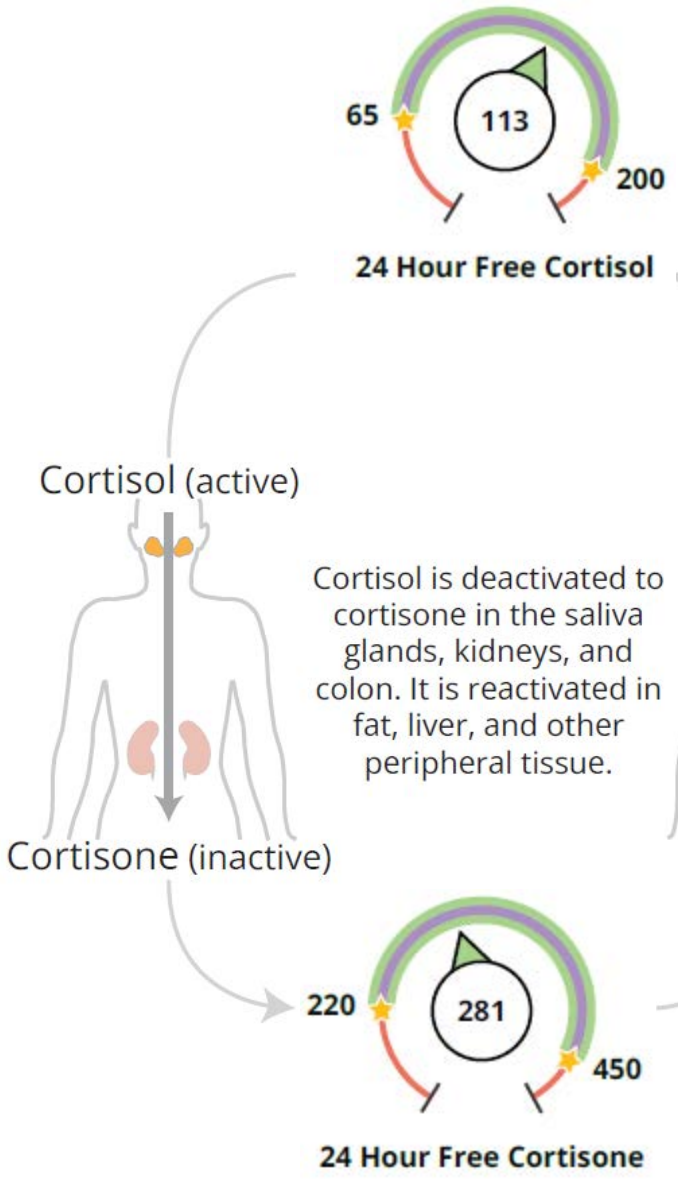




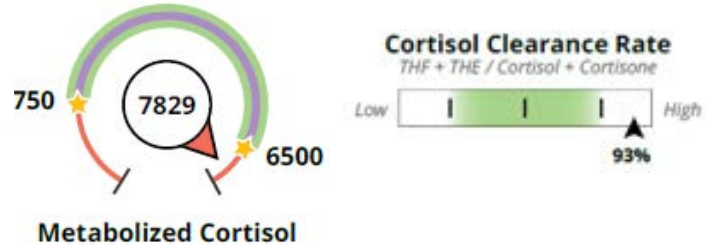
dutch®

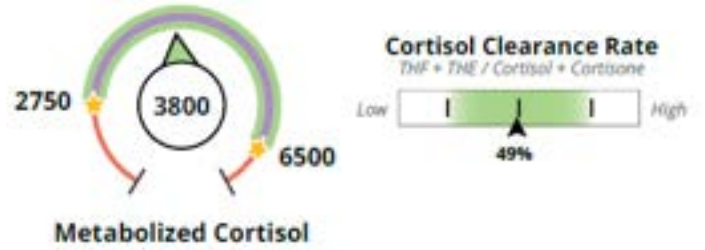


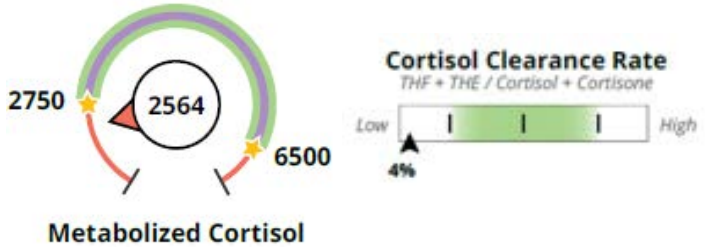




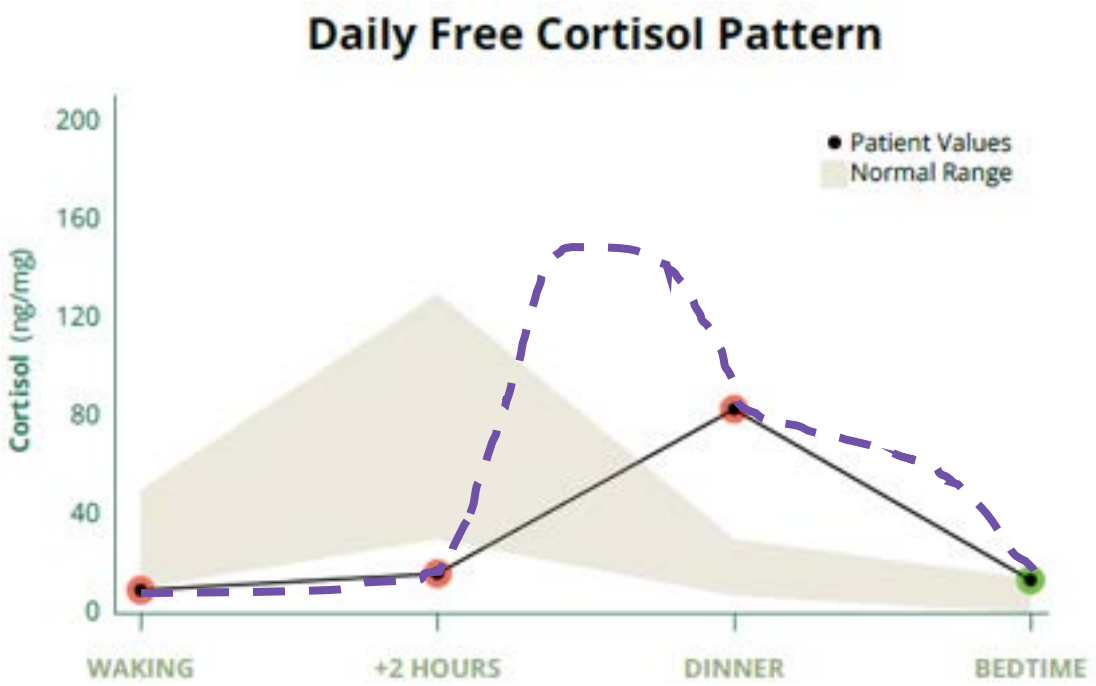
?

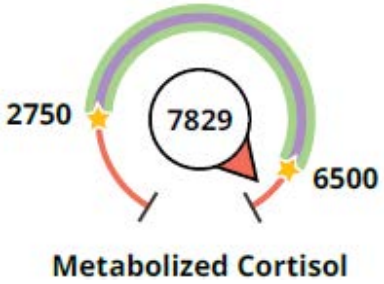




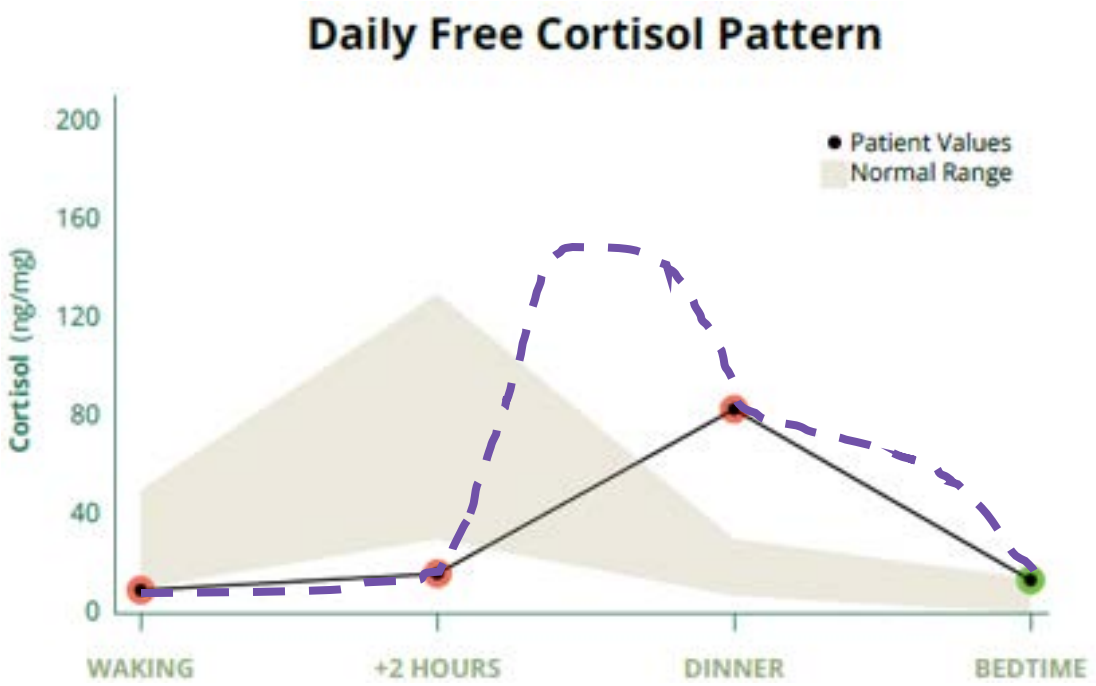


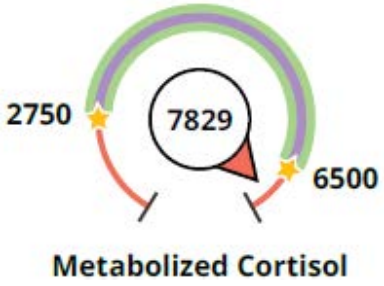
dutch®





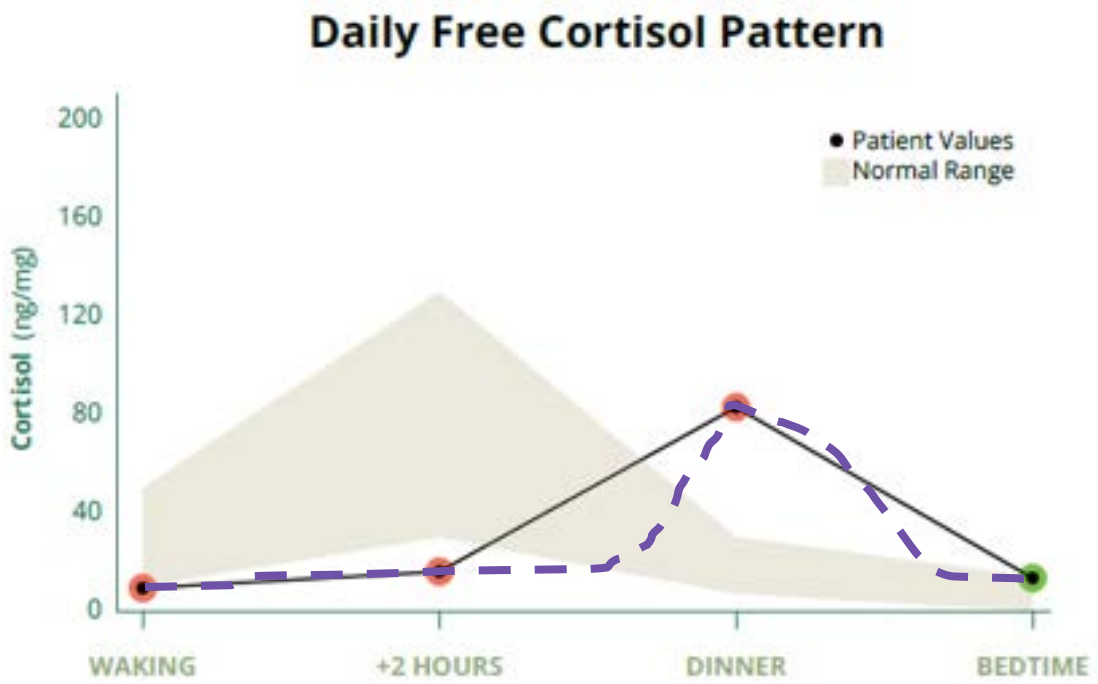
Interpretation when Free Cortisol >> Metabolized Cortisol?

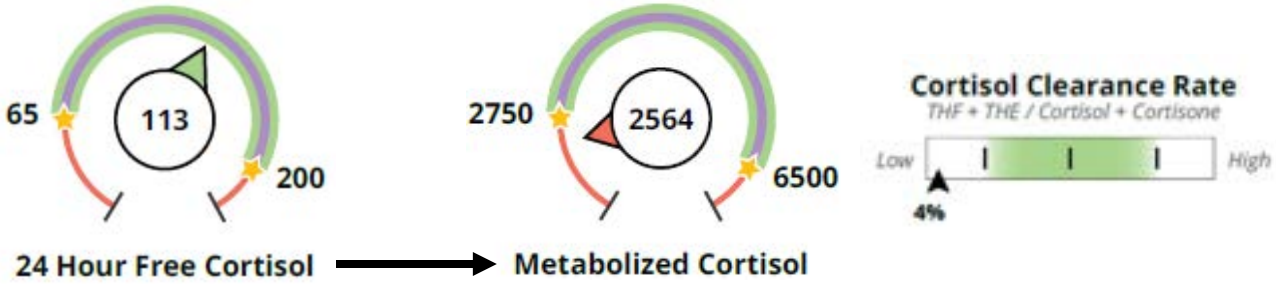




Interpretation Options

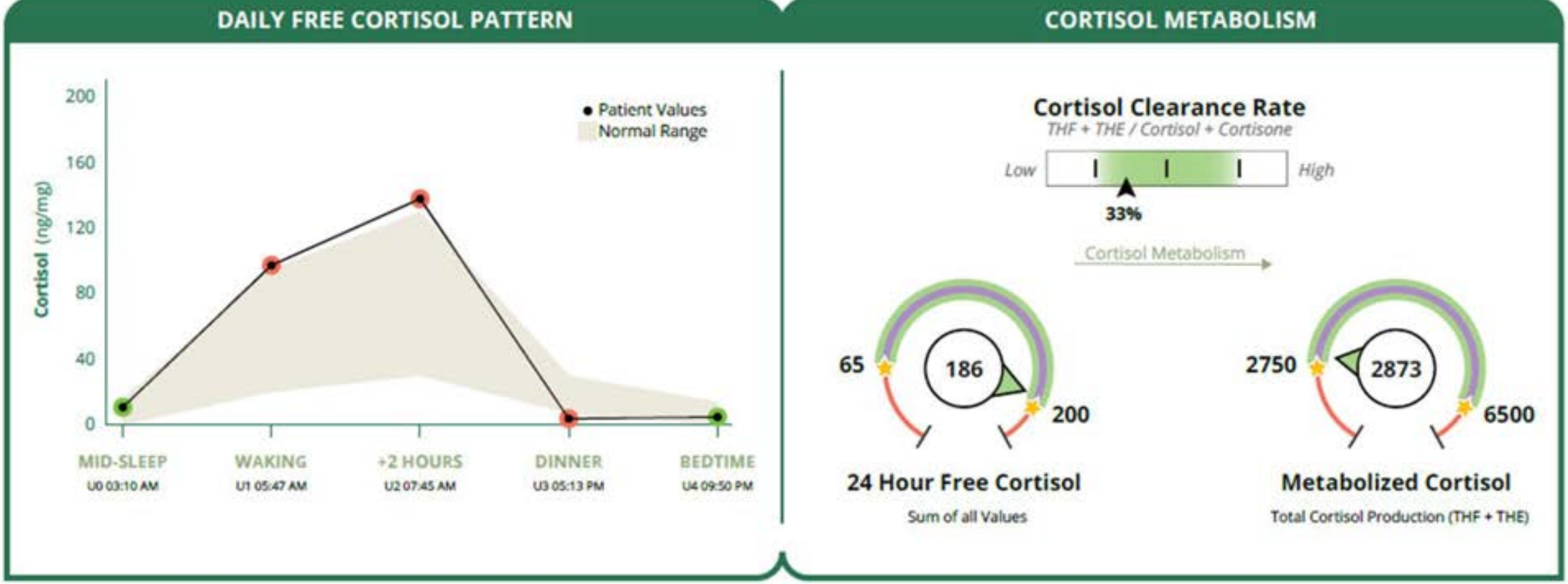
1. Cortisol clearance is accelerated – Why?
2. Cortisol production is higher than free cortisol implies.
   - Translation = free cortisol is high at times when samples are not collected.





Interpretation Options

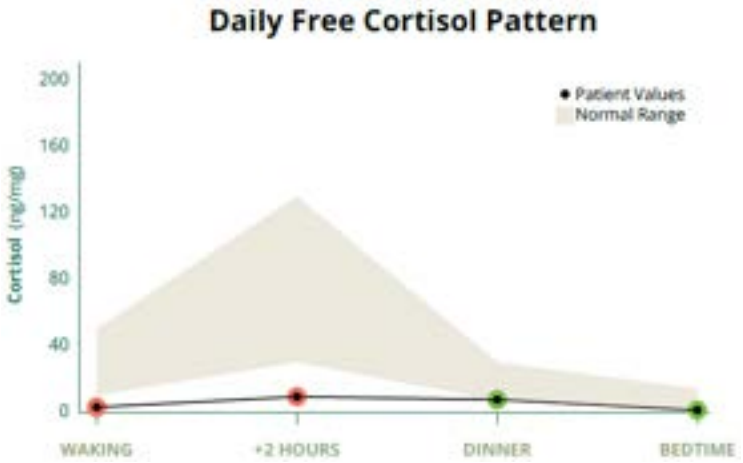
1. Cortisol clearance is “sluggish.” – Why?
2. Cortisol production is lower than free cortisol implies.
   - Translation = free cortisol is low at times when samples are not collected.

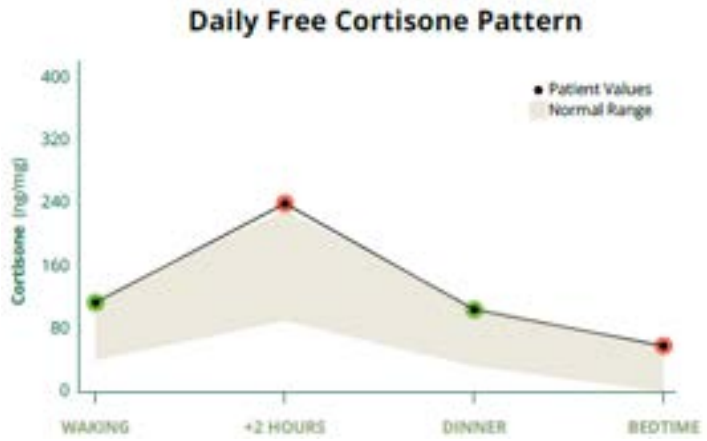


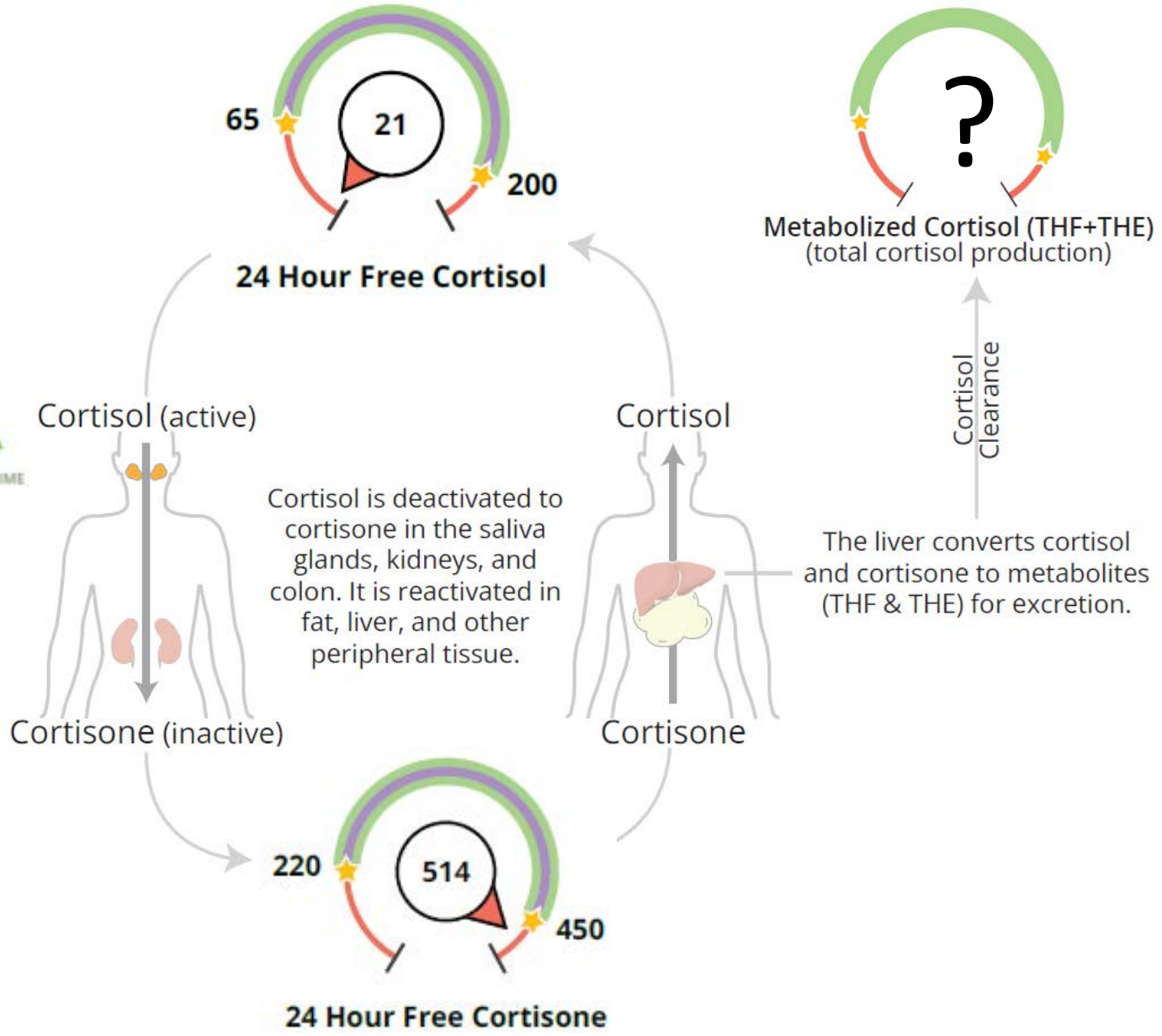
Interpretation Options

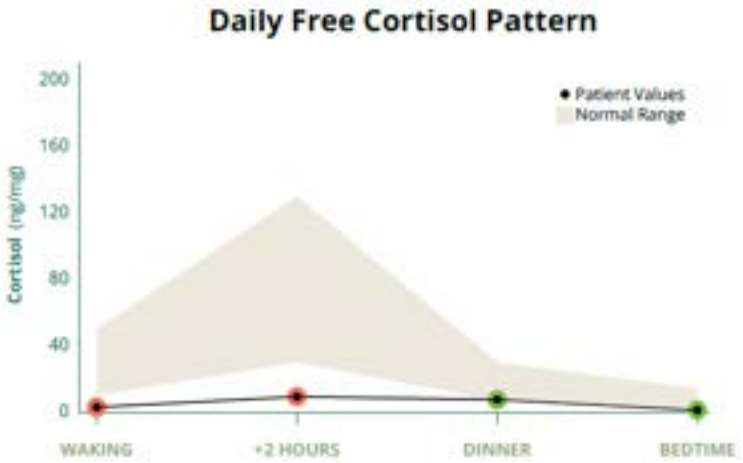
1. Cortisol clearance is “sluggish.” – Why?
2. Cortisol production is lower than free cortisol implies. What is happening 8am-3pm?
3. What if creatinine U1 and U2 are very low? What if only U3 has low creatinine?
4. What if cortisone is much lower than cortisol?

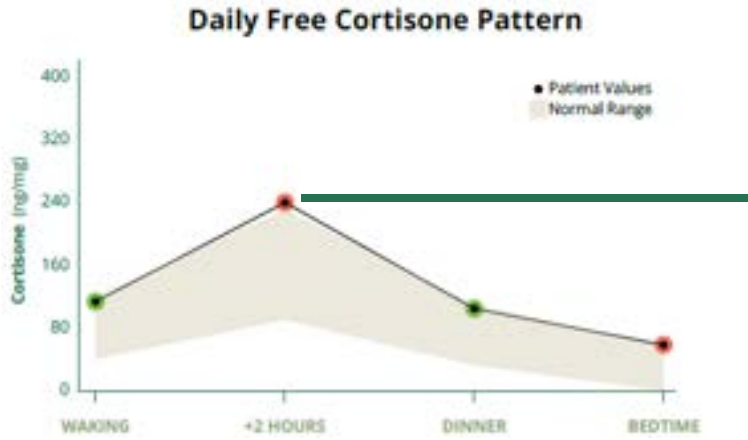
When Free Cortisol and Cortisone Don't Match

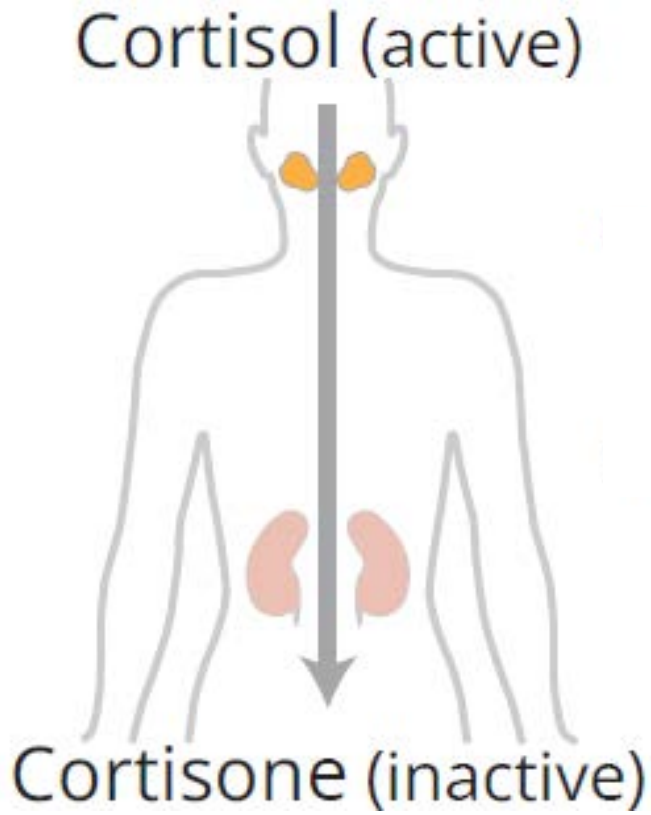




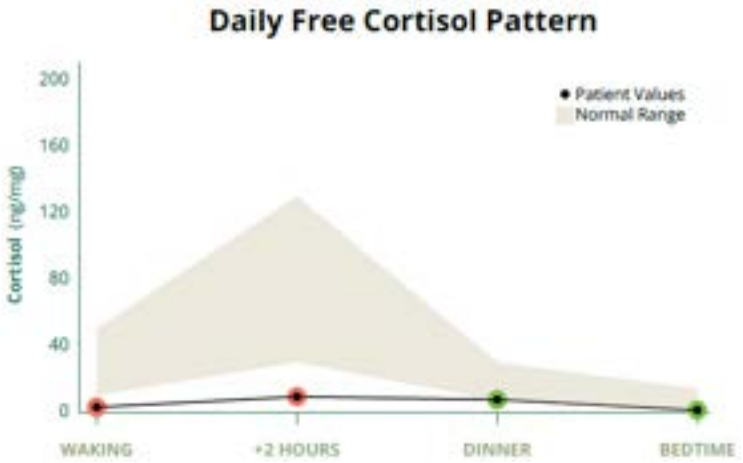


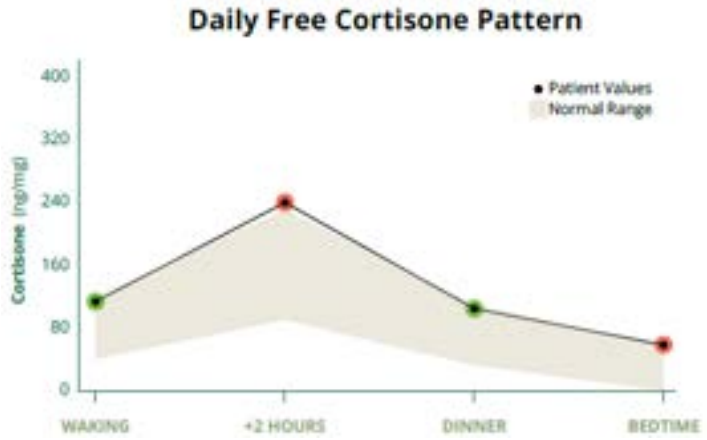






Most of this Cortisone entered the kidney as Cortisol





24 Hour Free Cortisol

Metabolized Cortisol (THF+THE)
(total cortisol production)

Cortisol Clearance

The liver converts cortisol and cortisone to metabolites (THF & THE) for excretion.

Cortisol (active)

Cortisol is deactivated to cortisone in the saliva glands, kidneys, and colon. It is reactivated in fat, liver, and other peripheral tissue.

Cortisone (inactive)

Cortisol

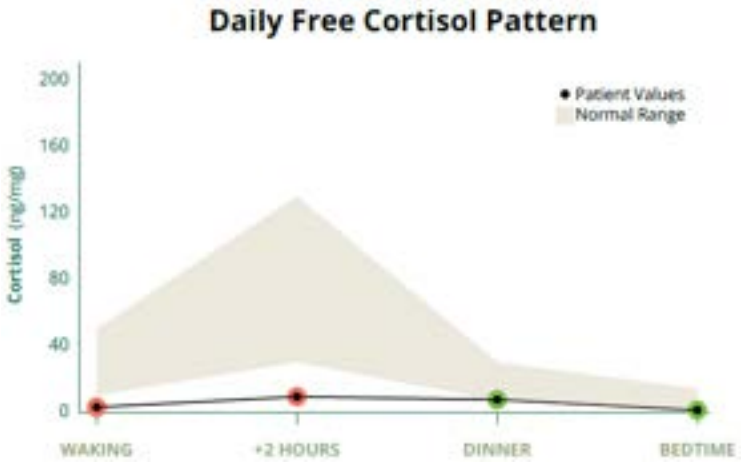
Cortisone

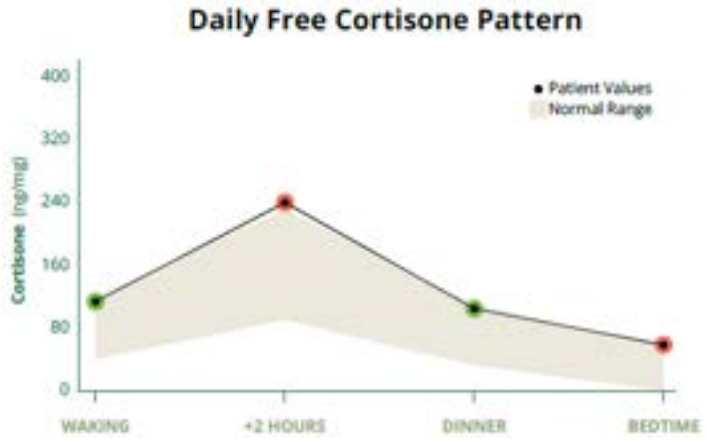
24 Hour Free Cortisone

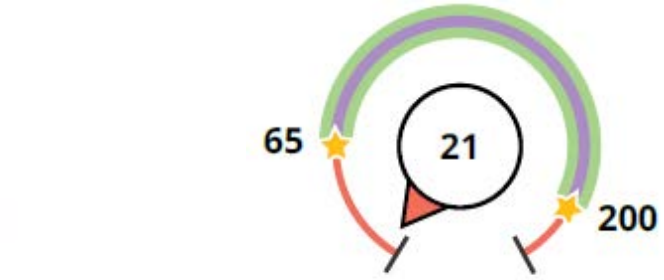
Systemic Preference

Cortisol / Cortisone Metabolites

dutch®







24 Hour Free Cortisol

Cortisol (active)

Cortisol is deactivated to cortisone in the saliva glands, kidneys, and colon. It is reactivated in fat, liver, and other peripheral tissue.

Cortisone (inactive)

24 Hour Free Cortisone

Cortisol

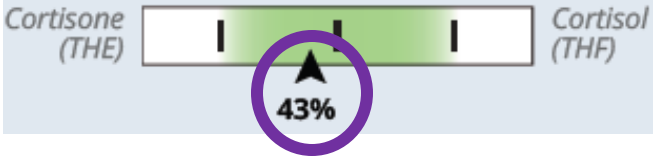
Cortisone

Metabolized Cortisol (THF+THE)
(total cortisol production)

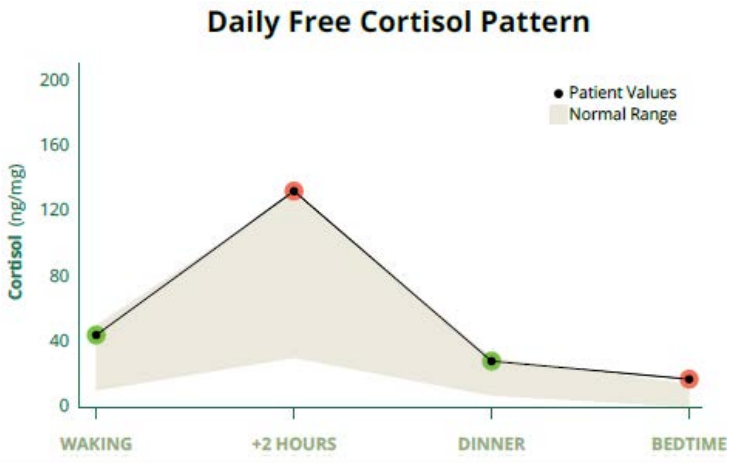
Cortisol Clearance

The liver converts cortisol and cortisone to metabolites (THF & THE) for excretion.

Systemic Preference
Cortisol / Cortisone Metabolites



dutch®



24 Hour Free Cortisol

Metabolized Cortisol (THF+THE)
(total cortisol production)

Cortisol Clearance

Cortisol (active)

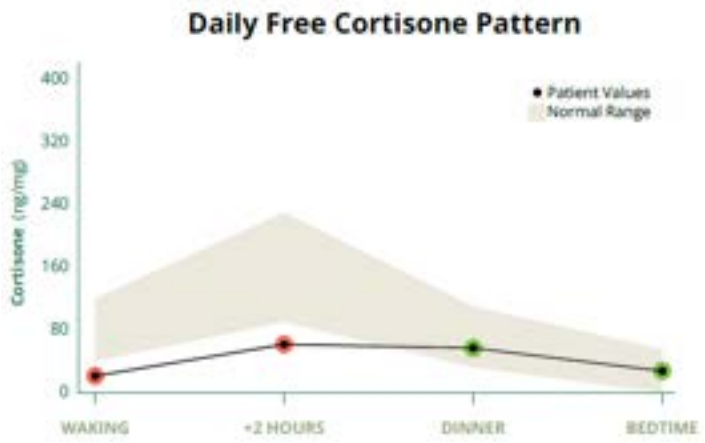
Cortisol

Cortisol is deactivated to cortisone in the saliva glands, kidneys, and colon. It is reactivated in fat, liver, and other peripheral tissue.

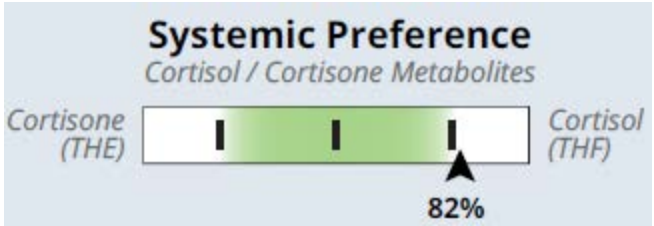
The liver converts cortisol and cortisone to metabolites (THF & THE) for excretion.

Cortisone (inactive)

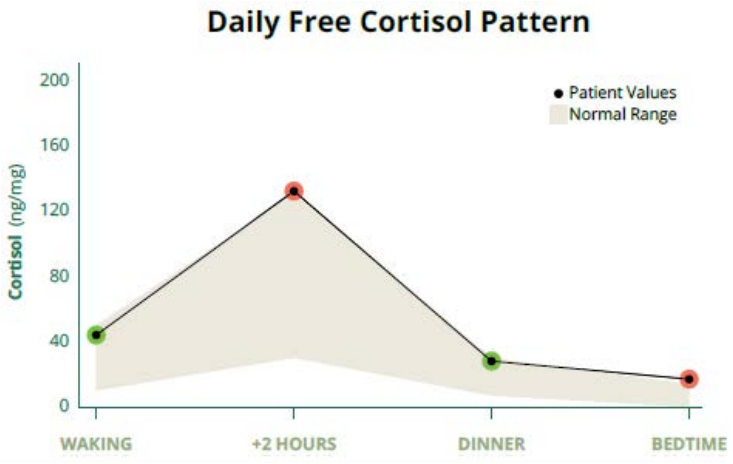
Cortisone

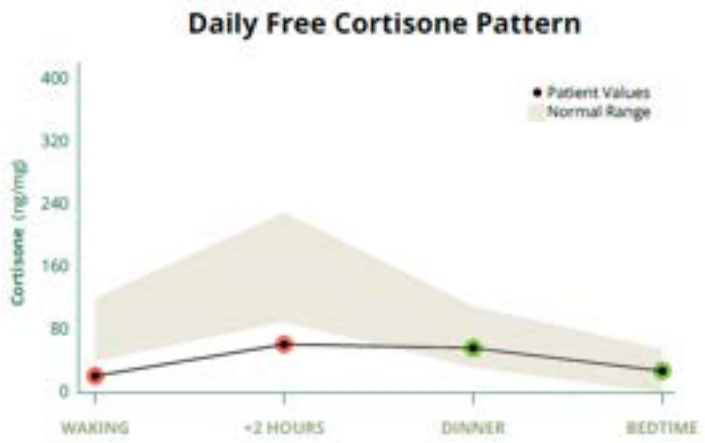


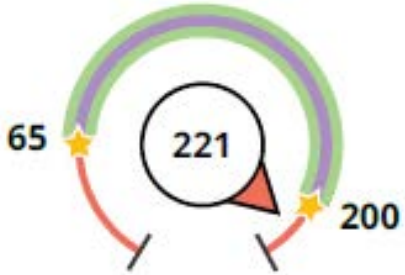
24 Hour Free Cortisone



dutch®







24 Hour Free Cortisol

Cortisol (active)

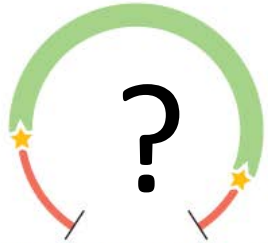
Cortisol is deactivated to cortisone in the saliva glands, kidneys, and colon. It is reactivated in fat, liver, and other peripheral tissue.

Cortisone (inactive)

24 Hour Free Cortisone

Cortisol

Cortisone



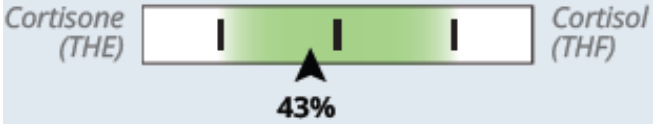
Metabolized Cortisol (THF+THE)
(total cortisol production)

Cortisol Clearance

The liver converts cortisol and cortisone to metabolites (THF & THE) for excretion.

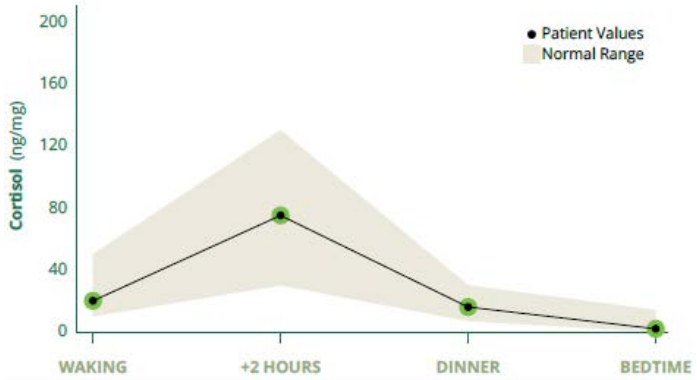
Systemic Preference

Cortisol / Cortisone Metabolites

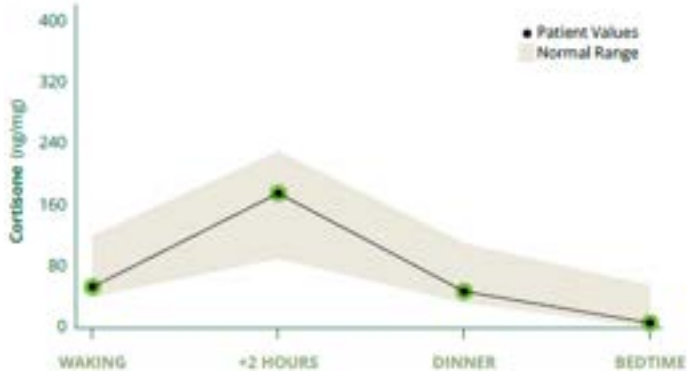


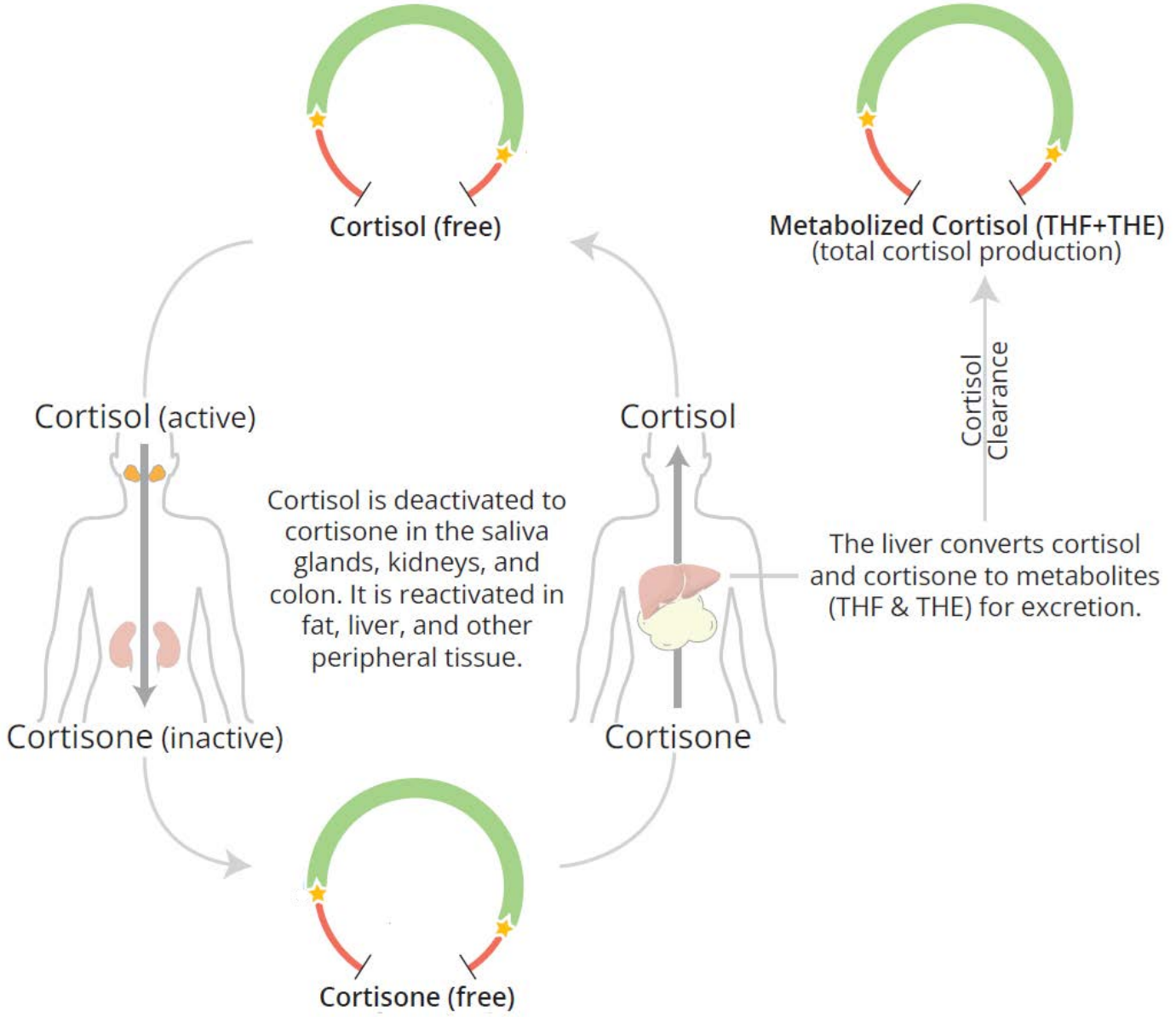
dutch®

Daily Free Cortisol Pattern



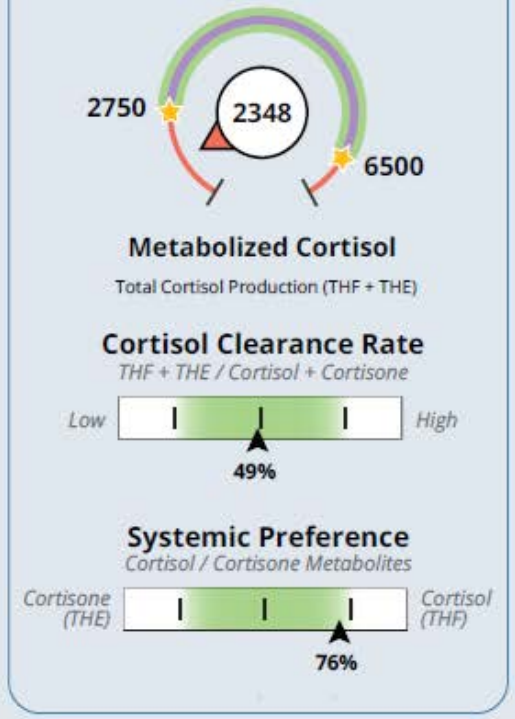
Daily Free Cortisone Pattern

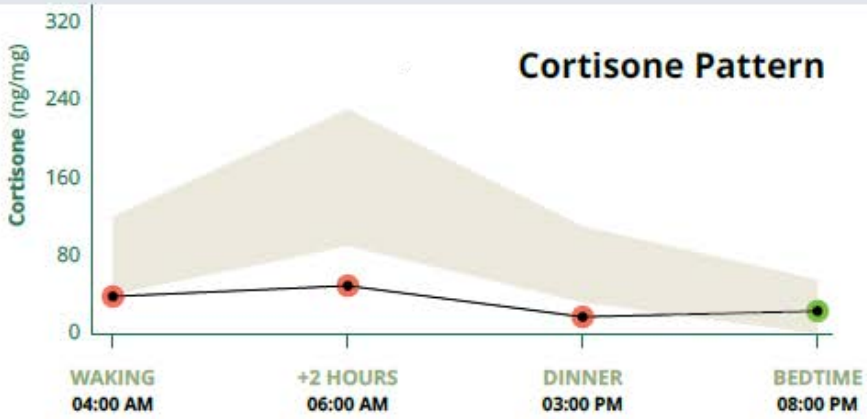


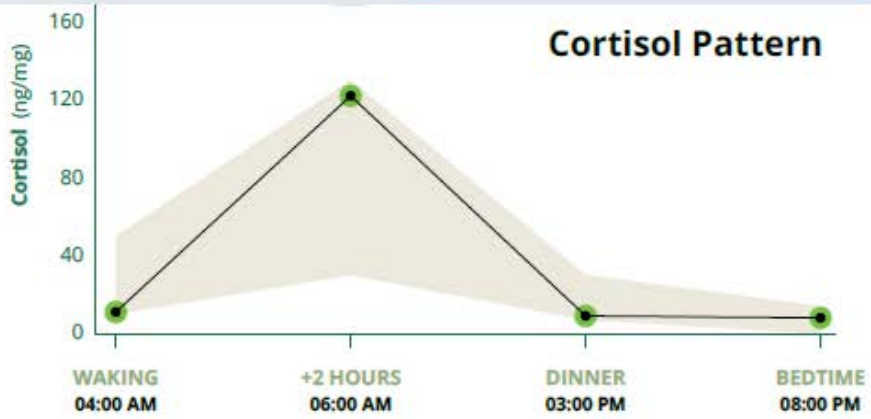


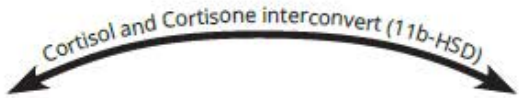
Interpretation Options

1. Cortisol overall is low except for an am surge helped by a lack of cortisol → cortisone (kidney)
   - Low creatinine in morning sample?
2. Morning surge not sustained very long?
   - am hydrocortisone use?
3. Overall preference is for cortisol
4. Symptom correlation?









Why Are We Here Today?

- Move beyond the DUTCH Test Summary Page
- Dig deeper into the DUTCH Test with Advanced Insights
- Understand common caveats to interpretation

Abnormal Results – Two Categories

From Yesterday

See it, Explain it

- Some abnormalities need to be understood but not fixed.

See it, Explain it, Fix it!

- Your job is to find abnormalities that need addressing.

Sometimes the DUTCH Test has more to offer

Move beyond the Summary Page to understand a patient's hormones at a deeper level

Beyond the Summary Page – Advanced Insights

A deeper understanding with NEW insights

Now you know something new!

A nuanced understanding of a Summary of Page conclusion

Now what you thought you knew is modified!

Today's Goals

- Understand common caveats to interpretation
- Move beyond the DUTCH Test Summary Page
- Dig deeper into the DUTCH Test with Advanced Insights

Thank You!

Mark Newman, DUTCH Founder

Questions?

mnewman@dutchtest.com

dutch®