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COMPLETE WITH CORTISOL AWAKENING RESPONSE

Accession # 00280395
Female Sample Report
123 A Street
Sometown, CA 90266

PRECISION ANALYTICAL INC.
SIMPLY · BETTER · TESTING

Last Menstrual Period:

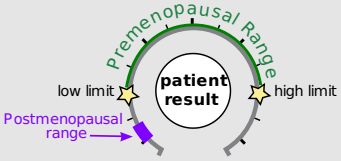
Ordering Physician:
Precision Analytical

DOB: 1976-01-01
Age: 41
Gender: Female

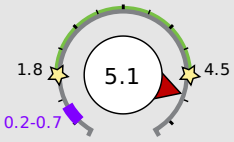
Collection Times:
2017-04-14 06:05AM (U)
2017-04-14 08:00AM (U)
2017-04-14 05:05PM (U)
2017-04-14 10:10PM (U)
2017-04-14 06:00AM (S)
2017-04-14 06:30AM (S)
2017-04-14 07:00AM (S)
2017-04-14 05:00PM (S)
2017-04-14 10:00PM (S)

Hormone Testing Summary

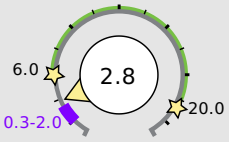
Key (how to read the results):



Sex Hormones
See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites

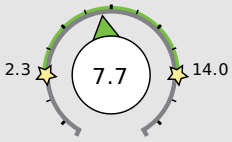


Estradiol(E2)



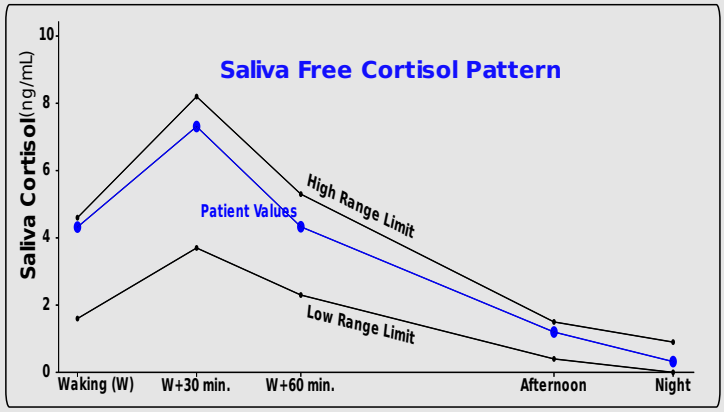
Progesterone
(Serum Equivalent, ng/mL)

Progesterone Serum Equivalent is a calculated value based on urine pregnanediol.



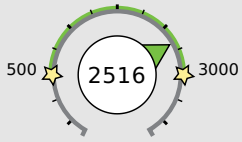
Testosterone

Adrenal Hormones
See pages 4 and 5 for a more complete breakdown of adrenal hormones



Total DHEA Production

Age	Range
20-39	1300-3000
40-59	750-2000
>60	500-1200



Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)

Saliva Cortisol Total
(Sum of 5 values)

cortisol metabolism →

Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

The following videos (which can also be found on the website under the listed names along with others) may aid your understanding:
DUTCH Plus Overview (quick overview) Estrogen Tutorial Female Androgen Tutorial Cortisol/CAR Tutorial

PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 8.

- The patient collected an "Insomnia" salivary sample in the middle of the night. The cortisol result for this sample was 2.10ng/mL (expected range 0-0.9). Please see page 4 for cortisol and cortisone results for this sample.
The Cortisol Awakening Response (CAR) was 2.99ng/mL (expected range 1.5-4.0) or 69.2% (range 50-160%). See page 5 for more details.

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COMPLETE WITH CORTISOL AWAKENING RESPONSE

Accession # 00280395
Female Sample Report
123 A Street
Sometown, CA 90266

PRECISION ANALYTICAL INC.
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Sex Hormones and Metabolites

Last Menstrual Period:

Ordering Physician:
Precision Analytical

DOB: 1976-01-01
Age: 41
Gender: Female

Collection Times:
2017-04-14 06:05AM (U)
2017-04-14 08:00AM (U)
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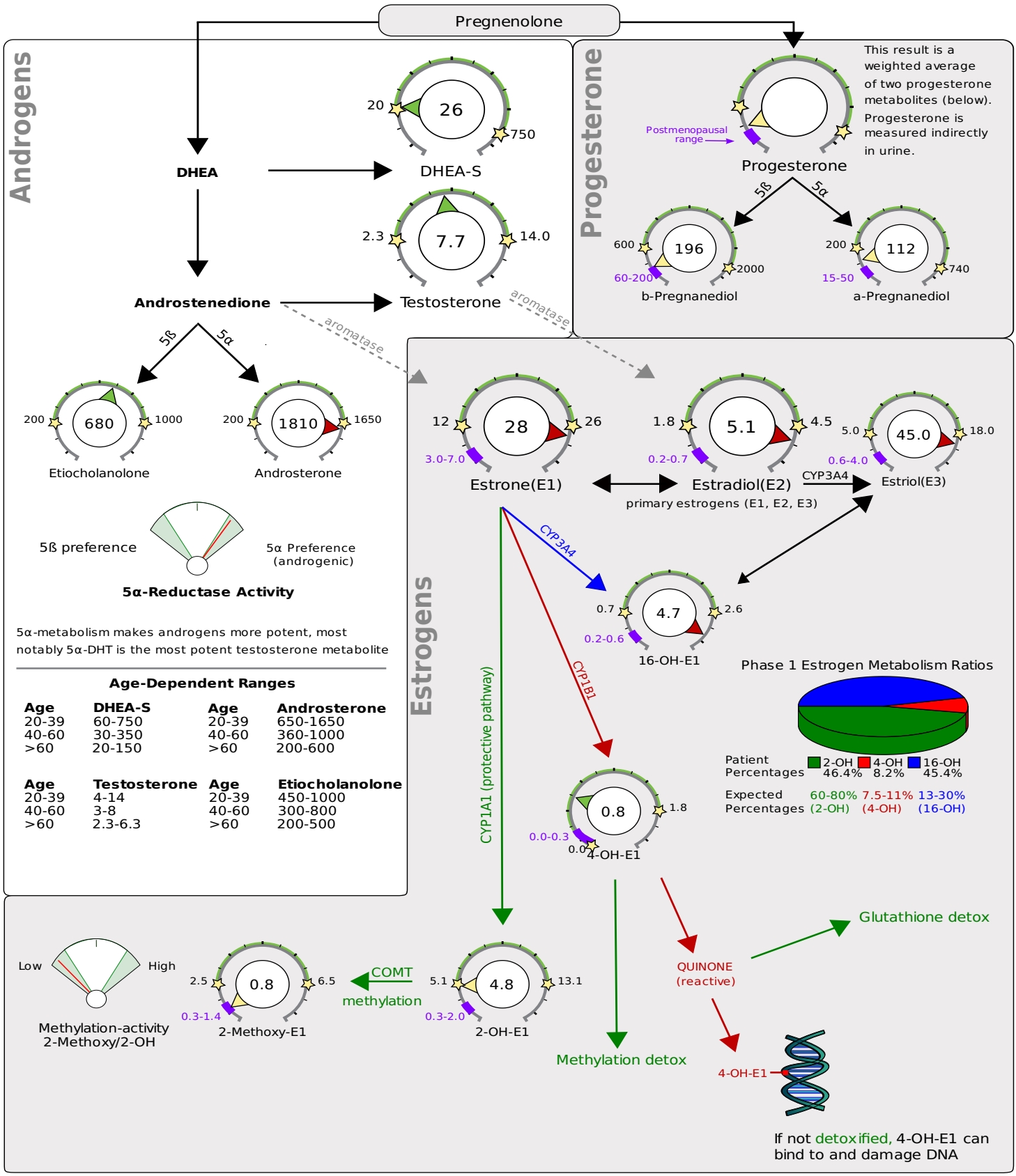
2017-04-14 06:00AM (S)
2017-04-14 06:30AM (S)
2017-04-14 07:00AM (S)
2017-04-14 05:00PM (S)
2017-04-14 10:00PM (S)

Test		Result	Units	Luteal* Range	Postmenopausal Range
Progesterone Metabolites (Urine)					
b-Pregnanediol	Below luteal range	196.0	ng/mg	600 - 2000	60-200
a-Pregnanediol	Below luteal range	112.0	ng/mg	200 - 740	15-50
Estrogens and Metabolites (Urine)					
Estrone(E1)	Above luteal range	28.2	ng/mg	12 - 26	3.0-7.0
Estradiol(E2)	Above luteal range	5.1	ng/mg	1.8 - 4.5	0.2-0.7
Estriol(E3)	Above luteal range	45.0	ng/mg	5 - 18	0.6-4.0
2-OH-E1	Below luteal range	4.8	ng/mg	5.1 - 13.1	0.3-2.0
4-OH-E1	Within luteal range	0.8	ng/mg	0 - 1.8	0-0.3
16-OH-E1	Above luteal range	4.7	ng/mg	0.7 - 2.6	0.2-0.6
2-Methoxy-E1	Below luteal range	0.8	ng/mg	2.5 - 6.5	0.3-1.4
2-OH-E2	Low end of luteal range	0.19	ng/mg	0 - 1.2	0-0.3
4-OH-E2	Within luteal range	0.2	ng/mg	0 - 0.5	0-0.1
2-Methoxy-E2	Within luteal range	0.4	ng/mg	0 - 0.7	0-0.4
Total Estrogen	Above range	89.79	ng/mg	35 - 70	4.0-15
Androgens and Metabolites (Urine)					
DHEA-S	Low end of range	26.0	ng/mg	20 - 750	
Androsterone	Above range	1810.0	ng/mg	200 - 1650	
Etiocholanolone	Within range	680.0	ng/mg	200 - 1000	
Testosterone	Within range	7.7	ng/mg	2.3 - 14	
5a-DHT	Above range	7.2	ng/mg	0 - 6.6	
5a-Androstanediol	Above range	42.0	ng/mg	12 - 30	
5b-Androstanediol	Within range	32.0	ng/mg	20 - 75	
Epi-Testosterone	Within range	8.8	ng/mg	2.3 - 14	

*the Luteal Range is the premenopausal range. When patients are taking oral progesterone this range for progesterone metabolites is not luteal and reflects the higher levels expected when patients take oral progesterone. This test is intended to be taken in the luteal phase of the menstrual cycle (days 19-22 of a 28 day cycle) for premenopausal women. The ranges in the table below may be used when samples are taken during the first few days (follicular) of the cycle, during ovulation (days 11-14) or when patients are on oral progesterone. See the following pages for age-dependent ranges for androgen metabolites.

Additional Normal Ranges	Follicular	Ovulatory	Oral Pg (100mg)
b-Pregnanediol	100-300	100-300	2000-9000
a-Pregnanediol	25-100	25-100	580-3000
Estrone (E1)	4.0-12.0	22-68	N/A
Estradiol (E2)	1.0-2.0	4.0-12.0	N/A

Hormone metabolite results from the previous page are presented here as they are found in the steroid cascade. See the Provider Comments for more information on how to read the results.



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COMPLETE WITH CORTISOL AWAKENING RESPONSE

Accession # 00280395
Female Sample Report
123 A Street
Sometown, CA 90266

PRECISION ANALYTICAL INC.
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Adrenal

Last Menstrual Period:

Ordering Physician:
Precision Analytical

DOB: 1976-01-01
Age: 41
Gender: Female

Collection Times:
2017-04-14 06:05AM (U)
2017-04-14 08:00AM (U)
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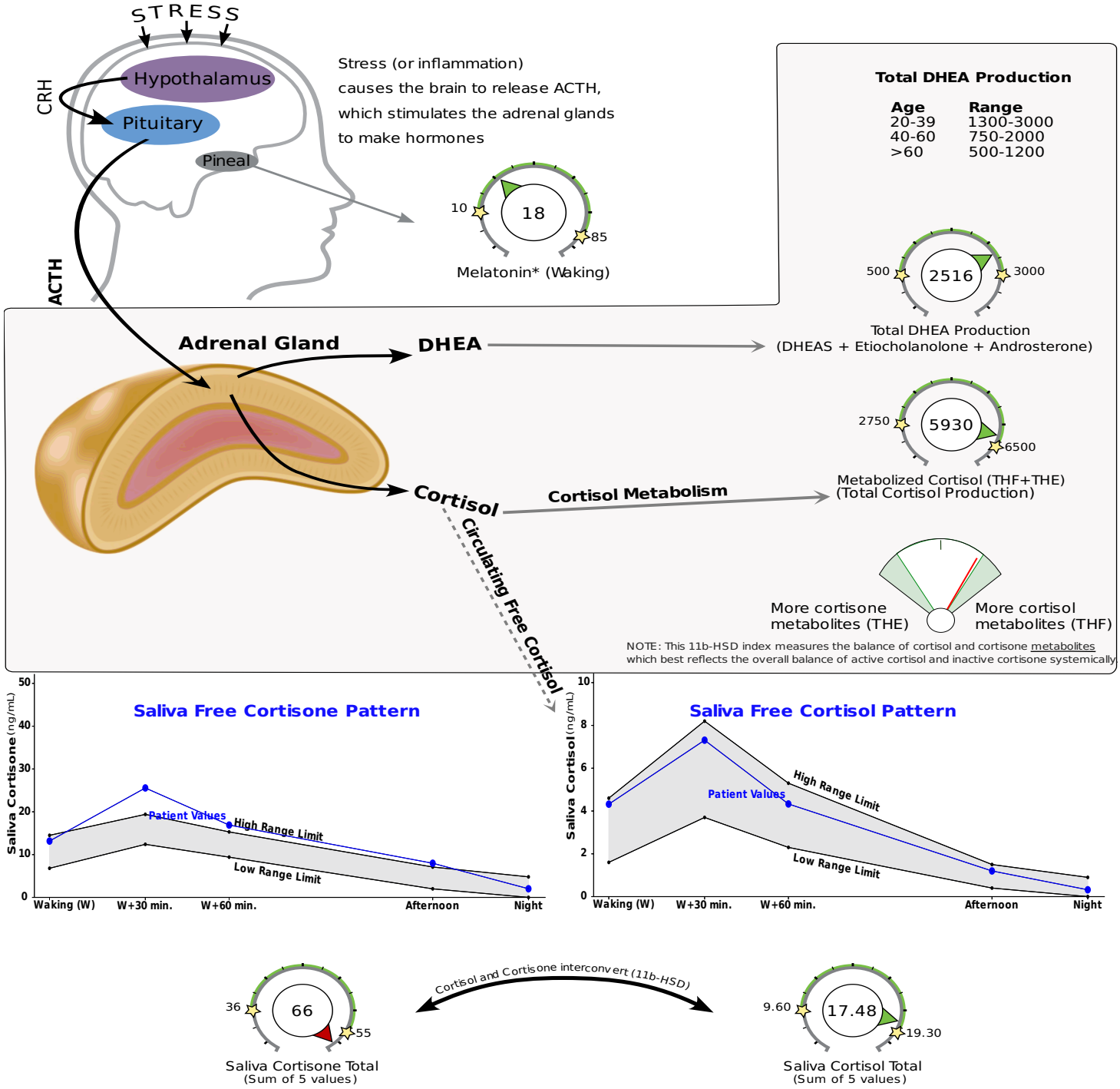
2017-04-14 07:00AM (S)
2017-04-14 05:00PM (S)
2017-04-14 10:00PM (S)
2017-04-14 01:30AM (S*)

Category	Test		Result	Units	Normal Range
Free Cortisol and Cortisone (Saliva)					
	Saliva Cortisol - Waking (W)	High end of range	4.32	ng/mL	1.6 - 4.6
	Saliva Cortisol - W+30 min.	High end of range	7.31	ng/mL	3.7 - 8.2
	Saliva Cortisol - W+60 min.	Within range	4.33	ng/mL	2.3 - 5.3
	Saliva Cortisol - Afternoon	Within range	1.2	ng/mL	0.4 - 1.5
	Saliva Cortisol - Night	Within range	0.32	ng/mL	0 - 0.9
	Saliva Cortisone - Waking (W)	High end of range	13.16	ng/mL	6.8 - 14.5
	Saliva Cortisone - W+30 min.	Above range	25.57	ng/mL	12.4 - 19.4
	Saliva Cortisone - W+60 min.	Above range	16.9	ng/mL	9.4 - 15.3
	Saliva Cortisone - Afternoon	Above range	7.98	ng/mL	2 - 7.1
	Saliva Cortisone - Night	Within range	2.02	ng/mL	0 - 4.8
	Saliva Cortisol Total	High end of range	17.48	ng/mL	9.6 - 19.3
	Saliva Cortisone Total	Above range	65.63	ng/mL	36 - 55
Creatinine (Urine)					
	Creatinine A (Waking)	Within range	0.4	mg/ml	0.2 - 2
	Creatinine B (Morning)	Within range	0.51	mg/ml	0.2 - 2
	Creatinine C (Afternoon)	Within range	0.92	mg/ml	0.2 - 2
	Creatinine D (Night)	Within range	1.01	mg/ml	0.2 - 2
Cortisol Metabolites and DHEA-S (Urine)					
	a-Tetrahydrocortisol (a-THF)	Above range	400.0	ng/mg	75 - 370
	b-Tetrahydrocortisol (b-THF)	Above range	2500.0	ng/mg	1050 - 2500
	b-Tetrahydrocortisone (b-THE)	Within range	3030.0	ng/mg	1550 - 3800
	Metabolized Cortisol (THF+THE)	High end of range	5930.0	ng/mg	2750 - 6500
	DHEA-S	Low end of range	26.0	ng/mg	20 - 750
Additional Cortisol and Cortisone (Saliva)					
*	Saliva Cortisol - Insomnia	Above range	2.1	ng/mL	0 - 0.9
*	Saliva Cortisone - Insomnia	Above range	10.4	ng/mL	0 - 4.8



- The patient submitted an Insomnia salivary sample. The cortisol result for this sample was 2.10ng/mL (expected range 0-0.9) The cortisone result for this sample was 10.4 ng/mL (expected range 0-4.8)

The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 4.32 and an increase to 7.3 after 30.0 minutes. This is an increase of 2.99ng/mL or 69.2%.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only. **This patient shows a salivary cortisol of 4.33 measured 60 minutes after waking. This is an increase of 0.01ng/mL or 0.23% compared to the waking sampe.** To date, data suggests that expected results may be 0-70%, and this guideline is considered for research only.

Accession # 00280395
Female Sample Report
123 A Street
Sometown, CA 90266

Organic Acid Tests (OATs)

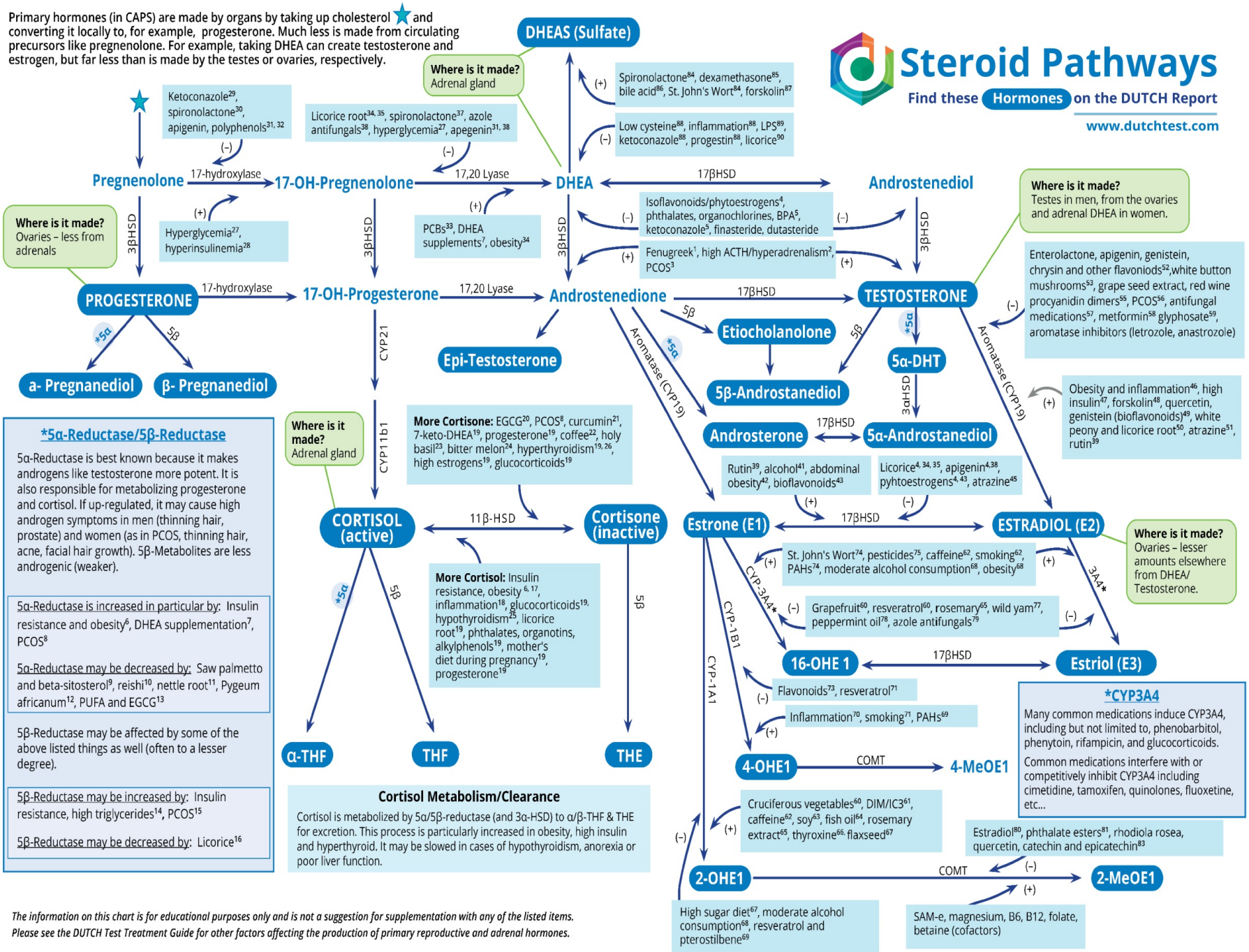
Last Menstrual Period:

Ordering Physician:
Precision Analytical

DOB: 1976-01-01
Age: 41
Gender: Female

Collection Times:
2017-04-14 06:05AM (U)
2017-04-14 08:00AM (U)
2017-04-14 05:05PM (U)
2017-04-14 10:10PM (U)
2017-04-14 06:00AM (S)
2017-04-14 06:30AM (S)
2017-04-14 07:00AM (S)
2017-04-14 05:00PM (S)
2017-04-14 10:00PM (S)

Category	Test		Result	Units	Normal Range
Nutritional Organic Acids					
Vitamin B12 Marker (may be deficient if high) - (Urine)					
	Methylmalonate (MMA)	Within range	1.2	ug/mg	0 - 2.2
Vitamin B6 Markers (may be deficient if high) - (Urine)					
	Xanthurenate	Above range	6.8	ug/mg	0 - 1.4
	Kynurenate	Above range	35.5	ug/mg	0 - 7.3
Glutathione Marker (may be deficient if low or high) - (Urine)					
	Pyroglutamate	Below range	23.2	ug/mg	32 - 60
Neurotransmitter Metabolites					
Dopamine Metabolite - (Urine)					
	Homovanillate (HVA)	Low end of range	5.6	ug/mg	4 - 13
Norepinephrine/Epinephrine Metabolite - (Urine)					
	Vanilmandelate (VMA)	Within range	4.8	ug/mg	2.4 - 6.4
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)					
	Melatonin* (Waking)	Low end of range	18.2	ng/mg	10 - 85
Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)					
	8-OHdG (Waking)	High end of range	4.3	ng/mg	0 - 5.2



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Female Sample Report
FINAL REPORT
02/24/2020

Precision Analytical (Raymond Grimsbo, Ph.D., Lab Director)
3138 Rivergate Street #301C
McMinnville, OR 97128

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Provider Notes

How to read the DUTCH report

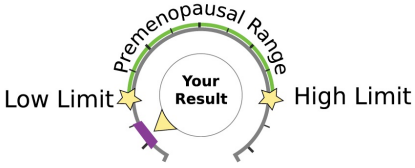
This report is not intended to treat, cure or diagnose any specific diseases. The graphic dutch dials in this report are intended for quick and easy evaluation of which hormones are out of range. Results below the left star are shaded yellow and are below range (left). Results between the stars and shaded green are within the reference range (middle). Results beyond the second star and shaded red are above the reference range (right). Some of these hormones also change with age, and the age-dependent ranges provided should also be considered.

Low Example

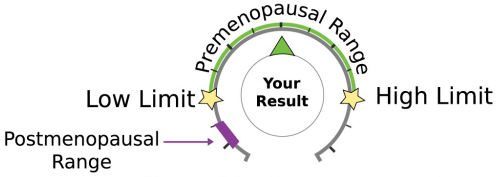
Normal Example

High Example

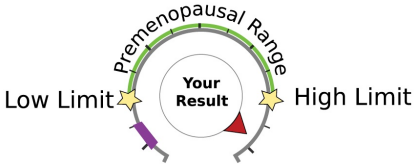
For female reproductive hormones, a purple band is present on the dutch dials. This band represents the expected levels (reference range) for postmenopausal (or non-cycling) women.



Low for Premenopausal
Normal for Postmenopausal

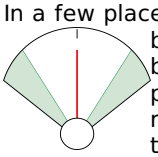


Normal for Premenopausal
High for Postmenopausal



High for Premenopausal
High for Postmenopausal

In a few places on the graphical pages, you will see fan-style gauges. For sex hormones, you will see one for the balance between 5a/5b metabolism as well as methylation. For adrenal hormones, you will see one to represent the balance between cortisol and cortisone metabolites. These indexes simply look at the ratio of hormones for a preference. An average or "normal" ratio between the two metabolites (or groups of metabolites) will give a result in the middle (as shown here). If the ratio between the metabolites measured is "low" the gauge will lean to the left and similarly to the right if the ratio is higher than normal.



Patient or Sample Comments

Throughout the provider comments you may find some comments specific to your situation or results. These comments will be found in this section or within another section as appropriate. Comments in other sections that are specific to your case will be in **bold**.

The patient reports regular menstrual cycles.

Note: The dates listed on the samples imply that they were older than our allowed 3 weeks when they were received. The instructions ask that patients freeze or refrigerate samples if they are to be held. If that is not the case, the free cortisol and cortisone levels may drop somewhat over time if the samples are too old. Other hormones tested are stable for more than 12 weeks at room temperature. Samples that are refrigerated or frozen are stable for months.

Progesterone Metabolism

The primary role of progesterone is to balance the strong effects of estrogen. Progesterone metabolites are measured and reflect progesterone levels well because very little progesterone is found in urine, so b-Pregnanediol is typically used as a surrogate marker because it is the most abundant metabolite, but we also test the corresponding a-pregnanediol. The average of the two metabolites is reported for progesterone. If levels are in the lower part of the reference range compared to estrogen levels, symptoms of too much estrogen may occur.
When ordering the DUTCH Complete, you will see Progesterone Serum Equivalent on the summary page 1. The urine metabolites of progesterone have been proven to correlate strongly enough to serum progesterone to provide this value. The correlation is the strongest for values within the premenopausal luteal range. Urine metabolites can at times result in somewhat higher serum equivalent results in the postmenopausal range. For this reason the postmenopausal Serum Equivalent range is slightly higher than typical serum ranges. NOTE: If progesterone is taken orally (also with sublingual), these metabolites are elevated from gut metabolism and results do NOT accurately reflect serum levels.

Progesterone levels show she is low for a cycling female in the luteal phase. It is important to check in with her about the timing of her test in relation to her cycle before interpreting this result. If she collected too early or too late, she may have missed her progesterone peak. Ask how she calculated the day to take the samples and when she began her menses after taking the test. If she collected at the right time, this indicates she has not ovulated in the past 5-7 days and may be experiencing anovulatory cycles. She may need support in her HPO axis communication and Chaste Tree berry throughout the month could be considered to help her cycle regularity and ovulation.

Estrogen Metabolism

When evaluating estrogen levels, it is important to assess the following:

- **The status (low, normal or high?) of estrogen production:**

Levels of the primary ovarian product, estradiol (the strongest estrogen), as well as "total estrogens" may be considered. For women not on HRT, consider the appropriate range (premenopausal or postmenopausal).

- **Phase I Metabolism:**

Estrogen is metabolized (primarily by the liver) down three phase I pathways. The 2-OH pathway is considered the safest because of the anti-cancer properties of 2-OH metabolites. Conversely, the 4-OH pathway is considered the most genotoxic as its metabolites can create reactive products that damage DNA. The third pathway, 16-OH creates the most estrogenic of the metabolites (although still considerably less estrogenic than estradiol) - 16-OH-E1. If overall estrogen levels are high, production of 16-OH-E1 may exacerbate high estrogen symptoms. Similarly, a woman with very low levels of estrogens, may have less low estrogen symptoms if 16-OH metabolism is preferred. For example Armamento-Villareal showed that a higher 2-OH-E1/16-OH-E1 ratio correlated to bone loss (a low estrogen symptom). Estriol is thought of as a safer (weaker) estrogen metabolite, but it is important to remember that estriol is actually 16-OH-E2, so generally patients that make a lot of the potentially protective/weak estriol may also make a lot of the estrogenic 16-OH-E1.
When evaluating phase I metabolism, it may be important to look at the ratios of the three metabolites to see which pathways are preferred relative to one another. It may also be important to compare these metabolites to the levels of the parent hormones (E1, E2). If the ratios of the three metabolites are favorable but overall levels of metabolites are much lower than E1 and E2, this may imply sluggish phase I clearance of estrogens, which can contribute to high levels of E1 and E2. Similarly, patients with excessive phase I metabolism may have low E1 and E2 levels because of high rates of clearance (as opposed to simply not making a lot of estrogen).
The pie chart will assist you in comparing the three pathway options of phase I metabolism compared to what is "normal." 2-OH metabolism can be increased by using products containing D.I.M. or I-3-C. These compounds are found (or created from) in cruciferous vegetables and are known for promoting this pathway.

Patients typically metabolize a much higher percentage of their estrogens down the more protective 2-OH pathway in phase 1 detoxification. Diindolylmethane (DIM) or Indole-3-Carbinol containing products can help move estrogens more efficiently down this pathway. Be aware that this typically lowers most of the other estrogens, including E1 and E2 as well. If the patients are taking or considering hormone replacement therapy, these products may be considered but a higher dose of estrogen may be needed for the same clinical effect if taken at the same time.

- **Methylation (part of phase II metabolism) of estrogens:**

After phase I metabolism, both 4-OH and 2-OH (not 16-OH) estrogens can be deactivated and eliminated by methylation. The methylation-activity index shows the patient's ratio of 2-Methoxy-E1 / 2-OH-E1 compared to what is expected. Low methylation can be caused by low levels of nutrients needed for methylation and/or genetic abnormalities (COMT, MTHFR). The COMT enzyme responsible for methylation requires magnesium and methyl donors. Deficiencies in folate or vitamin B6 or B12 can cause low levels of methyl donors. MTHFR genetic defects can make it more difficult for patients to make sufficient methyl donors. Genetic defects in COMT can make methylation poor even in the presence of adequate methyl donors.

Androgen Metabolism

When evaluating androgen levels, it is important to assess the following:

- **The status (low, normal or high?) of DHEA:**

DHEA and androstenedione are made almost exclusively by the adrenal gland (although a smaller amount is made in the ovaries). These hormones appear in urine as DHEA-S (DHEA-Sulfate), androsterone and etiocholanolone. The best way to assess the total production of DHEA is to add up these three metabolites. This total can be seen on the first page of the DUTCH Complete (and DUTCH Plus). DHEA production decreases quite significantly with age. Age-dependent ranges can be seen on the graphical page of results.

The Total DHEA Production (page 1) was about 2,516ng/mg which is within the overall range but is higher than expected for the patient's age (see the age-dependent ranges). Since these levels are normal for younger individuals, this may not necessarily be a bad thing. High DHEA can cause symptoms of androgen excess including oily skin, acne, sleep problems, headaches and mood disturbances. High levels may be due to supplementation, insulin, stress, elevated prolactin, alcohol and certain medications like ADD meds, Xanax and Wellbutrin. High DHEA can be treated with blood sugar balancing lifestyle, stress reduction and in appropriate cases ashwagandha. In some cases, highly androgenic people may show high levels of both DHEA or testosterone without negative clinical consequence.

Because inflammation blocks DHEA being converted to DHEAS, consider inflammation as a potential part of the overall clinical picture when DHEAS is significantly lower than the downstream metabolites of DHEA (Androsterone, Etiocholanolone) as seen in this case. A sulfur deficiency can also lead to adequate androgens but deficient DHEA-S.

- **The status (low, normal or high?) of testosterone:**

Females make most of their DHEA in the adrenal gland and a fraction of that DHEA trickles down metabolically to testosterone. For premenopausal women, some testosterone is also made by the ovaries. Levels of testosterone do drop somewhat with age, but not to the degree that DHEA decreases.

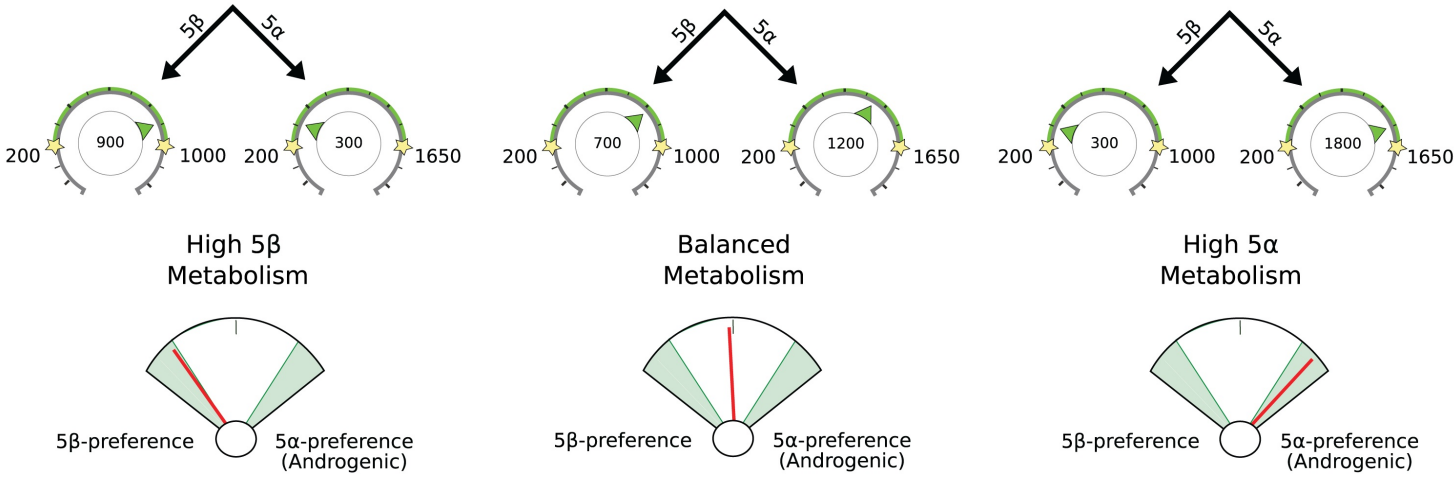
Testosterone levels for this patient were approximately 7.7ng/mg, which is within range. If symptoms

potentially related to high or low testosterone exist, you may also want to carefully evaluate 5a-metabolism (see below). You may also want to evaluate testosterone's downstream metabolites, 5a-androstanediol and 5b-androstanediol. These two metabolites generally parallel testosterone production, although they can also be generated from DHEA without going through testosterone.

- **The metabolic preference for the 5a (5-alpha) or 5b (5-beta) pathway:**

5a-reductase converts testosterone into 5a-DHT (DHT), which is even more potent (~3x) than testosterone. High levels of DHT can lead to symptoms associated with too much testosterone. Metabolites created down the 5b-pathway are significantly less androgenic than their 5a counterparts. In the examples below, the example on the left shows a patient with 5b-metabolism preference. A patient with a pattern like the example on the right may have high androgen symptoms even though the hormones are in the normal range because of the likely preference for turning a lot of her testosterone into DHT. The fan-style gauge below the hormones shows the 5a or 5b preference based on etiocholanolone (5b) and androsterone (5a) results. Progesterone metabolites are also metabolized by 5a and 5b enzymes and the balance between its two metabolites can be useful to confirm a 5a or 5b preference.

Example of how to read fan-style gauge for 5a-reductase activity:



While testosterone levels are not high, overall DHEA production is on the higher side and androgens are preferring the androgenic 5a pathway. Since the patient did not list significant symptoms of high androgens, these higher levels may be well tolerated by the patient. Since high insulin levels can lead to more DHEA production and 5a-metabolism, it may be worth exploring potential issues with blood sugar and/or insulin.

It is important to consider DHEA and testosterone production, 5a-metabolism patterns as well as the patient symptoms. For example, a woman with higher levels of DHEA and testosterone will often have high androgen symptoms (facial hair, thinning scalp hair, etc.) exacerbated by 5a-metabolism. If, on the other hand, she prefers 5b-metabolism she may not express high androgen symptoms in spite of higher levels of testosterone because 5b is the less androgenic pathway.
Testosterone levels may be better understood by also considering its downstream metabolites (5a-androstanediol, 5b-androstanediol). Technically, these metabolites can also be formed from DHEA metabolites without going through the testosterone pathway, but they generally tend to correlate with testosterone production.
You will also see levels of epi-testosterone, which is not androgenic like testosterone. It happens to be produced in about the same concentrations as testosterone (this is an approximate relationship). This can be helpful to assess testosterone therapy and rare cases where testosterone may have other complexities.

DUTCH Adrenal

The HPA-Axis refers to the communication and interaction between the hypothalamus (H) and pituitary (P) in the brain down to the adrenal glands (A) that sit on top of your kidneys. When a physical or psychological stressor occurs, the hypothalamus tells the pituitary to make ACTH, a hormone. ACTH stimulates the adrenal glands to make the stress hormone, cortisol and to a lesser extent DHEA and DHEA-S. Normally, the HPA-axis production follows a daily pattern in which cortisol rises rather rapidly in the first 10-30 minutes after waking (this is the C.A.R.) in order to help with energy, then gradually decreases throughout the day so that it is low at night for sleep. The cycle starts over the next morning. Abnormally high activity occurs in Cushing's Disease where the HPA-axis is hyper-stimulated causing cortisol to be elevated all day. The opposite is known as Addison's Disease, where cortisol is abnormally low because it is not made appropriately in response to ACTH's stimulation. These two conditions are somewhat rare. Examples of more common conditions related to less severely abnormal cortisol levels include fatigue, depression, insomnia, fibromyalgia, anxiety, inflammation and more.

Only a fraction of cortisol is "free" and bioactive. This fraction of cortisol is very important, but levels of metabolized cortisol best represent overall production of cortisol therefore both should be taken into account to correctly assess adrenal function.

When evaluating cortisol levels, it is important to assess the following:

- **The overall up-and-down pattern of free cortisol throughout the day, looking for low and high levels:**
Abnormal results should be considered along with related symptoms.
- **The sum of the free cortisol as an expression of the overall tissue cortisol exposure:**
This total of five free cortisol measurements is the best way to assess the total of free cortisol throughout the day, but do be aware that it is heavily weighted towards the morning production since three of five measurements are made within the first hour of the day.
- **The total level of cortisol metabolites:**
We call this calculation "Metabolized Cortisol" which is the sum of a-THF, b-THF and b-THE. While free cortisol is the best assessment for tissue levels of cortisol, it only represents 1-3% of the total produced. The majority of cortisol results in a urine metabolite and the total of these metabolites best represents the total glandular output of cortisol for the day. When overall production is much higher than free cortisol levels, cortisol clearance may be increased (as seen in hyperthyroidism, obesity, etc.) The most common reason for sluggish cortisol clearance (assumed when free cortisol levels are much higher than metabolized cortisol) is low thyroid.
- **A potential preference for cortisol or cortisone (the inactive form):**
Looking at the comparison between the total for free cortisol and free cortisone is NOT the best indication of a person's preference for cortisol or cortisone. The saliva gland converts cortisol to cortisone in the local tissue. This localized conversion can be seen by comparing cortisol and cortisone levels. To see the patient's preference systemically, it is best to look at which *metabolite* predominates (THF or THE). This preference can be seen in the gauge below metabolized cortisol. This is known as the 11b-HSD index. The enzyme 11b-HSD II converts cortisol to cortisone in the kidneys, saliva gland and colon. 11b-HSD I is more active in the liver, fat cells and the periphery and is responsible for reactivating cortisone to cortisol. Both are then metabolized by 5a-reductase to become tetrahydrocortisol (THF) and tetrahydrocortisone (THE) respectively.
- **The Cortisol Awakening Response (CAR):**
The unique feature of the DUTCH Plus is the inclusion of the CAR assessment. The response to waking adds one more piece to HPA-axis function. In some cases overall levels of free cortisol may be normal, but the response to stress may be under or overactive. Reasons for a lower CAR might include: an underactive HPA Axis, excessive psychological burnout, seasonal affective disorder (SAD), sleep apnea or poor sleep in general, PTSD, and "chronic fatigue" patients. An elevated CAR can be a result of an over-reactive HPA axis, ongoing job-related stress (anticipatory stress for the day), glycemic dysregulation, pain (ie. waking with painful joints or a migraine), and general depression (not SAD). Scientific literature points to the magnitude of the morning cortisol increase as being connected to HPA-axis health whether the overall production of cortisol is low, normal or high.

- The patient submitted an Insomnia salivary sample. The cortisol result for this sample was 2.10ng/mL. The cortisone result was 10.4 ng/mL. Ranges can be found in the table on the last page.

Nutritional Organic Acids

The following three organic acids are functional markers for vitamin deficiency. These compounds essentially back up in human biochemistry when a key nutrient is missing. These three metabolites have fairly straightforward interpretations. When the markers are elevated, it is likely that the patient's cellular levels of the related nutrient may be insufficient.

Methylmalonate (MMA)

Methylmalonate (also known as methylmalonic acid or MMA) is a functional marker of vitamin B12 (also known as cobalamin) deficiency. When cellular levels of B12 are low either from deficiency or due to a B12 transporter gene mutation, levels of MMA increase. This marker is considered superior to measuring serum B12 levels directly. A 2012 publication by Miller showed that 20% of those tested had a genetic defect in the protein that transports B12 to cells. These patients may have a functional B12 deficiency even if serum levels of B12 are normal.
If levels of MMA are elevated, it may be advisable to increase B12 consumption. Common foods high in B12 include beef liver, sardines, lamb, wild caught salmon, grass-fed beef, nutritional yeast and eggs. Vitamin B12 levels can also be increased through supplementation of B12 (taken as cobalamin, methylcobalamin, hydroxycobalamin, or adenosylcobalamin). Symptoms of a vitamin B12 deficiency include: fatigue, brain fog, memory problems, muscle weakness, unsteady gait, numbness, tingling, depression, migraines/headaches and low blood pressure.

Xanthurenate

Xanthurenate (also known as xanthurenic acid) and Kynurenate (kynurenic acid) are functional markers of vitamin B6 (also known as pyridoxine) deficiency. Vitamin B6 is a critical co-factor to over 100 important reactions that occur in the human body and is stored in the highest concentrations in muscle tissue. Tryptophan is readily converted to NAD by the liver. One of the steps in this pathway requires B6. When there is insufficient B6, xanthurenate is made instead. Kynurenate may also become elevated when patients are B6 deficient because of a different, possibly less B6 dependent pathway. The pathways leading to these biomarkers have other influences, so they will not always agree. When Xanthurenate is elevated, Kynurenate is also elevated about 1/3 of the time. When both are elevated, a B6 deficiency is likely more certain and more severe.
Not only is xanthurenate an indicator of a lack of B6, it is also harmful to the human body. It complexes with insulin and decreases insulin sensitivity. In fact, rats fed xanthurenate will actually develop diabetes because of the effects on insulin. If xanthurenate levels are elevated, B6 supplementation may be considered. Food high in B6 include turkey breast, grass-fed beef, pinto beans, avocado, pistachios, chicken, sesame and sunflower seeds.
While there is always some tryptophan going down the kynurenine pathway towards NAD (and possibly xanthurenate), this process is up-regulated by inflammation, estrogen and cortisol. If levels of estrogen or cortisol are high, it may exacerbate xanthurenate elevations and increase the need for B6.
Xanthurenate can also bind to iron and create a complex that increases DNA oxidative damage resulting in higher 8-OHdG levels. If both markers are elevated, there is likely an antioxidant insufficiency.

Xanthurenate and Kynurenate are both elevated in this case, so a vitamin B6 deficiency is likely and may be somewhat significant (since both markers are elevated). It is advisable to consider increasing vitamin B6 intake and to be aware of those things listed above that may induce a vitamin B6 deficiency.

Pyroglutamate

Pyroglutamate (also known as pyroglutamic acid) is a functional marker of glutathione deficiency. Pyroglutamate is a step in the production/recycling of glutathione. If the body cannot convert pyroglutamate forward, it will show up elevated in the urine. High pyroglutamate is an established marker for glutathione deficiency. Pyroglutamate in the urine can also be elevated with cheese consumption.
Glutathione is one of the most potent anti-oxidants in the human body. It is especially important in getting rid of toxins, including the reactive quinone species formed by 4-OH-E1 and 4-OH-E2. This reactive species can damage DNA if not detoxified by either methylation or glutathione.
Some have reported that low pyroglutamate may also be indicative of a need for glutathione; however, this is not established in the scientific literature.

Neurotransmitter Metabolites

The neurotransmitters dopamine, norepinephrine and serotonin are important for human health. Measuring neurotransmitters directly (direct testing of serotonin, for example) is difficult because of their instability and their urinary measurements are controversial with respect to how well they reflect the body's levels of these neuro-hormones. Each of these three neurotransmitters can be assessed indirectly by measuring their urine metabolites. While these metabolites are not a perfect reflection of what's going on in the brain, the scientific literature does affirm their use for a good representation of overall levels of these neurotransmitters.

Homovanillate (HVA)

Homovanillate (also known as HVA) is the primary metabolite of dopamine, a brain and adrenal neurotransmitter that comes from tyrosine (with BH4 and iron as co-factors) and goes on to create norepinephrine (noradrenaline) and epinephrine (adrenaline).
Low levels of HVA can be due to low levels of dopamine or poor conversion of dopamine to HVA. The latter may be due to insufficient levels of SAM, Magnesium, FAD and NAD which are needed to metabolize dopamine. Low circulating dopamine may be due to insufficient BH4, iron or tyrosine. It may also be seen when adrenal function is generally low. Low dopamine levels may be associated with addictions, cravings and pleasure seeking (to boost levels) in addition to sleepiness, impulsivity, tremors, less motivation, fatigue and low mood.
Elevated HVA may be caused by generally increased adrenal hormone output or because of a copper or vitamin C deficiency (which are needed for dopamine conversion to norepinephrine). Elevations may also be caused by a number of medications or supplements including: MAO inhibitors, quercetin, tyrosine, DL-phenylalanine (DLPA), L-dopa, macuna, dopamine medication (Levodopa, Sinemet, Methyldopa), SNRI medication (Wellbutrin), tricyclic antidepressants, amphetamines, appetite suppressants, and caffeine. Bananas also contain dopamine. Elevated dopamine may be associated with loss of memory, insomnia, agitation, hyperactivity, mania, hyper-focus, high stress and anxiety as well as addictions, cravings and pleasure seeking (to maintain high levels).
When HVA is very high, consider if the previously discussed foods, supplements or medications may be the cause. Rarely, tumors associated with increased HVA may be present. In these cases, further testing is necessary for diagnosis. High HVA alone is not diagnostic of a tumor.

Vanilmandelate (VMA)

Vanilmandelate (also known as VMA) is the primary metabolite of norepinephrine and epinephrine (adrenaline). The adrenal gland makes cortisol and DHEA as well as norepinephrine and epinephrine. When adrenal hormone output is generally low, VMA levels may be low. If HVA levels are significantly higher than VMA, there may be a conversion problem from dopamine to norepinephrine. This case can be caused by a copper or vitamin C deficiency. The enzymes COMT (methylation) and MAO are needed to make VMA from norepinephrine. If these enzymes are not working properly, VMA may be low when circulating norepinephrine and/or epinephrine are not low. Low levels of norepinephrine and epinephrine may be associated with addictions, cravings, fatigue, low blood pressure, low muscle tone, intolerance to exercise, depression, loss of alertness.
When the body is under physical or psychological stress, VMA levels may increase. Because dopamine gets converted to norepinephrine and ultimately to VMA, the list of medications and supplements that increase HVA may also increase VMA. Elevated levels may be associated with feeling stressed, aggression, violence, impatience, anxiety, panic, worry, insomnia, paranoia, increased tingling/burning, loss of memory, pain sensitivity, high blood pressure and heart palpitations.
If VMA and HVA are both extremely high, it may be necessary to rule out a neuroblastic tumor.

Melatonin (measured as 6-OHMS)

Melatonin is not technically an adrenal or sex hormone however it is highly involved in the entire endocrine system. It is made in small amounts in the pineal gland in response to darkness and stimulated by Melanocyte Stimulating Hormone (MSH). A low MSH is associated with insomnia, an increased perception of pain, and mold exposure. Pineal melatonin (melatonin is also made in significant quantities in the gut) is associated with the circadian rhythm of all hormones (including female hormone release). It is also made in small amounts in the bone marrow, lymphocytes, epithelial cells and mast cells. Studies have shown that a urine sample collected upon waking has levels of 6-Hydroxymelatonin-sulfate (6-OHMS) that correlate well to the total levels of melatonin in blood samples taken continuously throughout the night. The DUTCH test uses the waking sample only to test levels of melatonin production.
Low melatonin levels may be associated with insomnia, poor immune response, constipation, weight gain or increased appetite. Elevated melatonin is usually caused by ingestion of melatonin through melatonin supplementation or eating melatonin-containing foods. Elevated melatonin production that is problematic is rare, but levels can be higher in patients with Chronic Fatigue Syndrome and may be phase shifted (peaking later) in some forms of depression.

8-OHdG (8-Hydroxy-2-deoxyguanosine)

8-OHdG (8-hydroxy-2-deoxyguanosine) results can be seen on page 6 of the DUTCH Complete (or DUTCH Plus) report. It is a marker for estimating DNA damage due to oxidative stress (ROS creation). 8-OHdG is considered pro-mutagenic as it is a biomarker for various cancer and degenerative disease initiation and promotion. It can be increased by chronic inflammation, increased cell turnover, chronic stress, hypertension, hyperglycemia/pre-diabetes/diabetes, kidney disease, IBD, chronic skin conditions (psoriasis/eczema), depression, atherosclerosis, chronic liver disease, Parkinson's (increasing levels with worsening stages), Diabetic neuropathy, COPD, bladder cancer, or insomnia. Studies have shown higher levels in patients with breast and prostate cancers. When levels are elevated it may be prudent to eliminate or reduce any causes and increase the consumption of antioxidant containing foods and/or supplements.

The reference range for 8-OHdG is a more aggressive range for Functional Medicine that puts the range limit at the 80th percentile for each gender. A classic range (average plus two standard deviations) would result in a range of 0-6ng/mg for women and 0-10ng/mg for men. Seeking out the cause of oxidative stress may be more crucial if results exceed these limits.

Reference Range Determination (last updated 12.20.2018)

We aim to make the reference ranges for our DUTCH tests as clinically appropriate and useful as possible. This includes the testing of thousands of healthy individuals and combing through the data to exclude those that are not considered "healthy" or "normal" with respect to a particular hormone. As an example, we only use a premenopausal woman's data for estrogen range determination if the associated progesterone result is within the luteal range (days 19-21 when progesterone should be at its peak). We exclude women on birth control or with any conditions that may be related to estrogen production. Over time the database of results for reference ranges has grown quite large. This has allowed us to refine some of the ranges to optimize for clinical utility. The manner in which a metabolite's range is determined can be different depending on the nature of the metabolite. For example, it would not make clinical sense to tell a patient they are deficient in the carcinogenic estrogen metabolite, 4-OH-E1 therefore the lower range limit for this metabolite is set to zero for both men and women. Modestly elevated testosterone is associated with unwanted symptoms in women more so than in men, so the high range limit is set at the 80th percentile in women and the 90th percentile for men. Note: the 90th percentile is defined as a result higher than 90% (9 out of 10) of a healthy population.

Classic reference ranges for disease determination are usually calculated by determining the average value and adding and subtracting two standard deviations from the average, which defines 95% of the population as being "normal." When testing cortisol, for example, these types of two standard deviation ranges are effective for determining if a patient might have Addison's (very low cortisol) or Cushing's (very high cortisol) Disease. Our ranges are set more tightly to be optimally used for Functional Medicine practices.

Below you will find a description of the range for each test:

Female Reference Ranges (Updated 06.20.2019)

	Low%	High%	Low	High		Low%	High%	Low	High
b-Pregnanediol	20%	90%	600	2000	Saliva Cortisol Waking (W)	20%	90%	1.6	4.6
a-Pregnanediol	20%	90%	200	740	Saliva Cortisol (W+30 min.)	20%	90%	3.7	8.2
Estrone (E1)	20%	80%	12	26	Saliva Cortisol (W+60 min.)	20%	90%	2.3	5.3
Estradiol (E2)	20%	80%	1.8	4.5	Saliva Cortisol (Afternoon)	20%	90%	0.4	1.5
Estriol (E3)	20%	80%	5	18	Saliva Cortisol (Night)	0	95%	0	0.9
2-OH-E1	20%	80%	5.1	13.1	Saliva Cortisol (2-3 am)	0	90%	0	0.9
4-OH-E1	0	80%	0	1.8	Saliva Cortisone Waking (W)	20%	90%	6.8	14.5
16-OH-E1	20%	80%	0.7	2.6	Saliva Cortisone (W+30 min.)	20%	90%	12.4	19.4
2-Methoxy-E1	20%	80%	2.5	6.5	Saliva Cortisone (W+60 min.)	20%	90%	9.4	15.3
2-OH-E2	0	80%	0	1.2	Saliva Cortisone Afternoon	20%	90%	2	7.1
4-OH-E2	0	80%	0	0.5	Saliva Cortisone Night	0	95%	0	4.8
2-Methoxy-E2	0	80%	0	0.7	Saliva Cortisone (2-3 am)	0	95%	0	4.8
DHEA-S	20%	90%	20	750	Melatonin (6-OHMS)	20%	90%	10	85
Androsterone	20%	80%	200	1650	8-OHdG	0	90%	0	5.2
Etiocholanolone	20%	80%	200	1000	Methylmalonate	0	90%	0	2.2
Testosterone	20%	80%	2.3	14	Xanthurenate	0	90%	0	1.4
5a-DHT	20%	80%	0	6.6	Kynurenate	0	90%	0	7.3
5a-Androstanediol	20%	80%	12	30	Pyroglutamate	10%	90%	32	60
5b-Androstanediol	20%	80%	20	75	Homovanillate	10%	95%	4	13
Epi-Testosterone	20%	80%	2.3	14	Vanilmandelate	10%	95%	2.4	6.4
a-THF	20%	90%	75	370					
b-THF	20%	90%	1050	2500	**Calculated Values**				
b-THE	20%	90%	1550	3800	Total DHEA Production	20%	80%	500	3000
% = population percentile: Example - a high limit of 90% means results higher than 90% of the women tested for the reference range will be designated as "high."					Total Estrogens	20%	80%	35	70
					Metabolized Cortisol	20%	90%	2750	6500
					Saliva Cortisol Total	20%	90%	9.6	19.3
					Saliva Cortisone Total	20%	90%	36	55

Provider Notes:

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