



Accession # 00526631
Jane Doe 4



Last Menstrual Period:

2023-05-21

Collection Times:

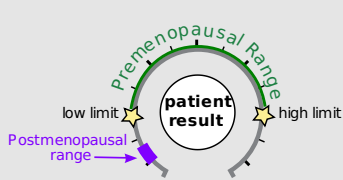
2023-06-08 01:15AM
2023-06-08 04:35AM
2023-06-08 06:35AM
2023-06-08 04:30PM
2023-06-08 09:08PM

Ordering Provider:
Precision Analytical

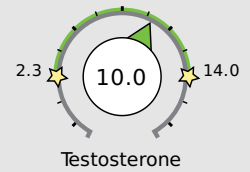
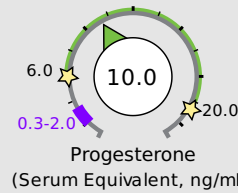
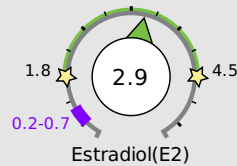
DOB: 1986-01-01
Age: 37
Sex: Female

Hormone Testing Summary

Key (how to read the results):

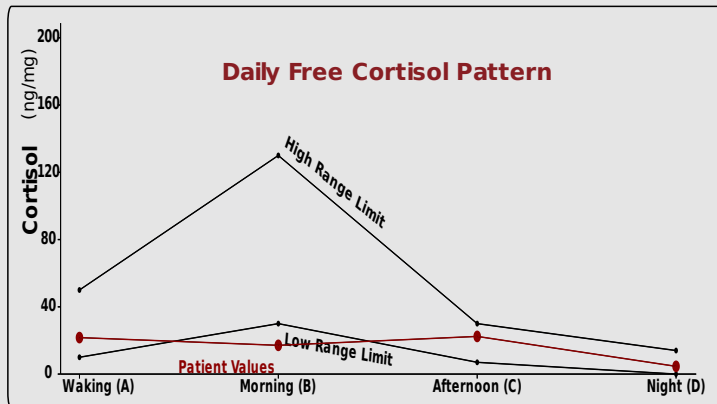


Sex Hormones See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites



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

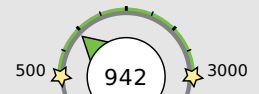
Adrenal Hormones See pages 4 and 5 for a more complete breakdown of adrenal hormones



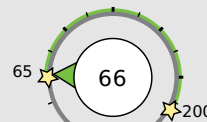
Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

Total DHEA Production

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

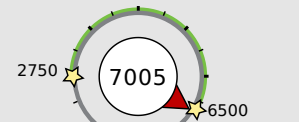


Total DHEA Production (DHEAS + Etiocholanolone + Androsterone)



24hr Free Cortisol (A+B+C+D)

cortisol metabolism



Metabolized Cortisol (THF+THE) (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 Complete Overview](#) [Estrogen Tutorial](#) [Female Androgen Tutorial](#) [Cortisol Tutorial](#)

PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 9.

- Note that the Progesterone Serum Equivalent is not a valid concept when patients are taking oral or sublingual progesterone. Swallowed progesterone creates very high levels of the metabolites measured and these do not necessarily parallel serum progesterone.
- The patient reports using a glucocorticoid. This may suppress adrenal function. See provider comments for more details.



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Sex Hormones and Metabolites

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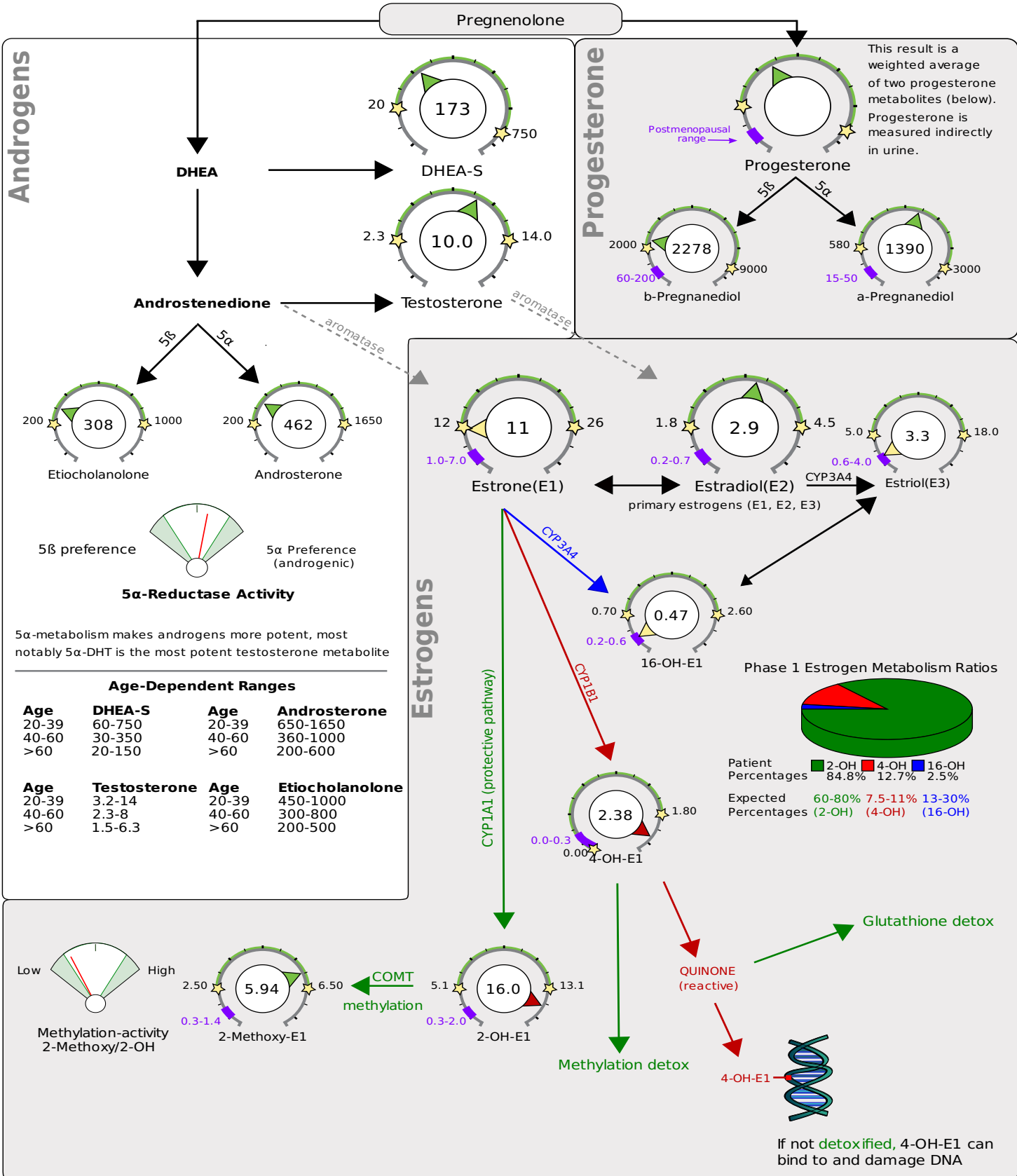
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Test	Result	Units	Luteal* Range	Postmenopausal Range
Progesterone Metabolites (Urine)				
b-Pregnanediol	Low end of range	2278.0	ng/mg 2000 - 9000	60-200
a-Pregnanediol	Within range	1390.3	ng/mg 580 - 3000	15-50
Estrogens and Metabolites (Urine)				
Estrone(E1)	Below luteal range	11.3	ng/mg 12 - 26	1.0-7.0
Estradiol(E2)	Within luteal range	2.9	ng/mg 1.8 - 4.5	0.2-0.7
Estriol(E3)	Below luteal range	3.3	ng/mg 5 - 18	0.6-4.0
2-OH-E1	Above luteal range	16.0	ng/mg 5.1 - 13.1	0.3-2.0
4-OH-E1	Above luteal range	2.38	ng/mg 0 - 1.8	0-0.3
16-OH-E1	Below luteal range	0.47	ng/mg 0.7 - 2.6	0.2-0.6
2-Methoxy-E1	High end of luteal range	5.94	ng/mg 2.5 - 6.5	0.3-1.4
2-OH-E2	High end of luteal range	1.01	ng/mg 0 - 1.2	0-0.3
4-OH-E2	Above luteal range	0.96	ng/mg 0 - 0.5	0-0.1
Total Estrogen	Within range	44.3	ng/mg 35 - 70	4.0-15
Androgens and Metabolites (Urine)				
DHEA-S	Within range	172.9	ng/mg 20 - 750	
Androsterone	Low end of range	462.0	ng/mg 200 - 1650	
Etiocholanolone	Low end of range	308.0	ng/mg 200 - 1000	
Testosterone	Within range	10.0	ng/mg 2.3 - 14	
5a-DHT	Low end of range	1.31	ng/mg 0 - 6.6	
5a-Androstenediol	High end of range	26.0	ng/mg 6 - 30	
5b-Androstenediol	Low end of range	30.3	ng/mg 20 - 75	
Epi-Testosterone	Within range	9.8	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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Adrenal

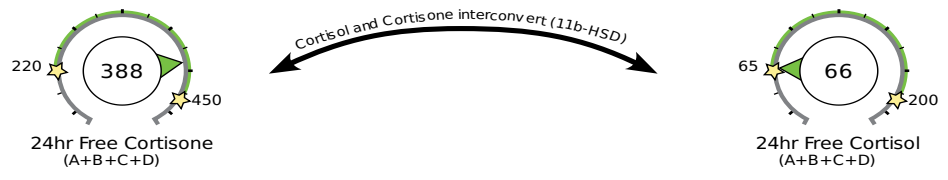
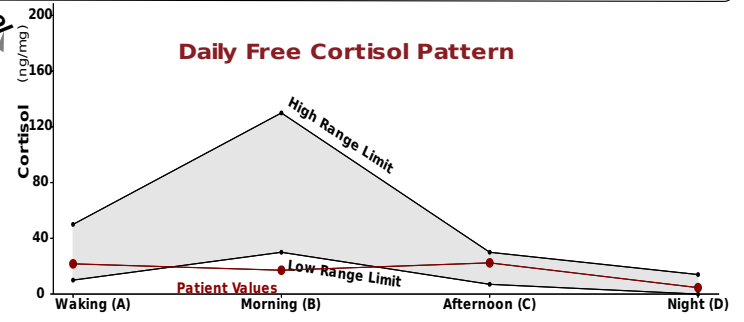
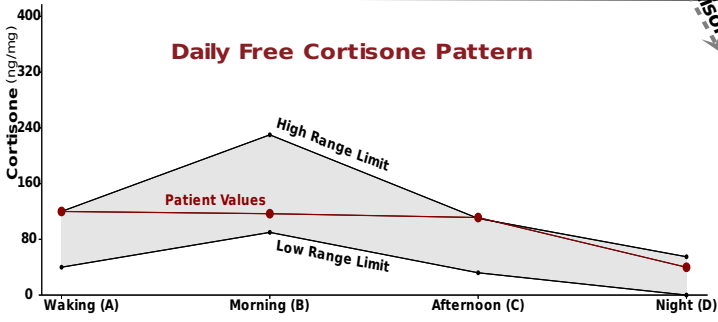
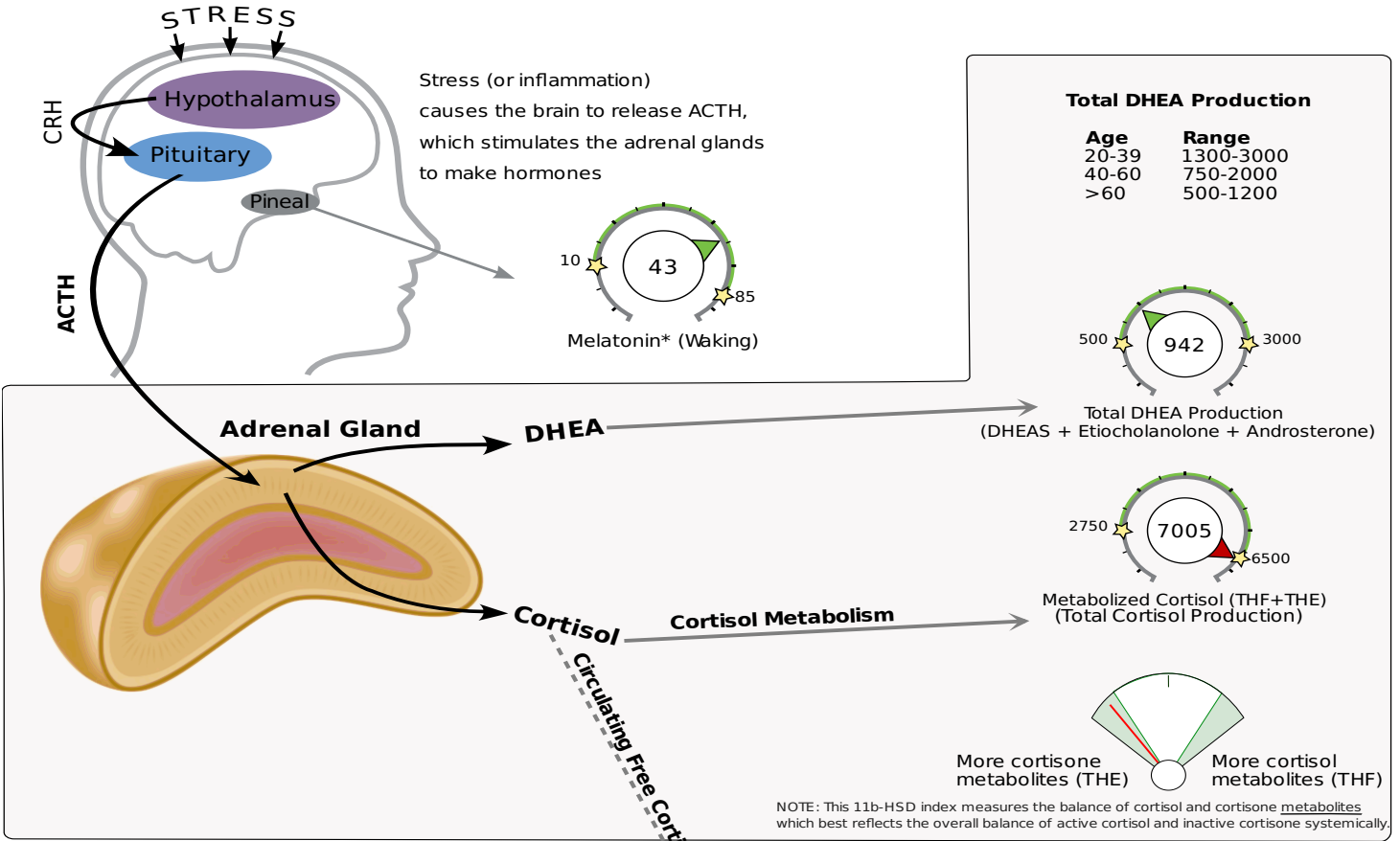
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Category	Test		Result	Units	Normal Range
Creatinine (Urine)					
	Creatinine A (Waking)	Within range	0.8	mg/ml	0.2 - 2
	Creatinine B (Morning)	Within range	0.92	mg/ml	0.2 - 2
	Creatinine C (Afternoon)	Above range	2.14	mg/ml	0.2 - 2
	Creatinine D (Night)	Above range	2.21	mg/ml	0.2 - 2
Daily Free Cortisol and Cortisone (Urine)					
	Cortisol A (Waking)	Within range	21.7	ng/mg	10 - 50
	Cortisol B (Morning)	Below range	17.1	ng/mg	30 - 130
	Cortisol C (Afternoon)	Within range	22.4	ng/mg	7 - 30
	Cortisol D (Night)	Within range	4.6	ng/mg	0 - 14
	Cortisone A (Waking)	Above range	120.0	ng/mg	40 - 120
	Cortisone B (Morning)	Low end of range	116.8	ng/mg	90 - 230
	Cortisone C (Afternoon)	Above range	111.3	ng/mg	32 - 110
	Cortisone D (Night)	Within range	39.9	ng/mg	0 - 55
	24hr Free Cortisol	Low end of range	66.0	ng/mg	65 - 200
	24hr Free Cortisone	Within range	388.0	ng/mg	220 - 450
Cortisol Metabolites and DHEA-S (Urine)					
	a-Tetrahydrocortisol (a-THF)	Within range	244.0	ng/mg	75 - 370
	b-Tetrahydrocortisol (b-THF)	High end of range	2271.0	ng/mg	1050 - 2500
	b-Tetrahydrocortisone (b-THE)	Above range	4490.0	ng/mg	1550 - 3800
	Metabolized Cortisol (THF+THE)	Above range	7005.0	ng/mg	2750 - 6500
	DHEA-S	Within range	173.0	ng/mg	20 - 750



The first value reported (Waking "A") for cortisol is intended to represent the "overnight" period. When patients sleep through the night, they collect just one sample. In this case, the patient woke during the night and collected (see the top of the report for the times collected). We call this value "A1" and the value from the sample collected at waking "A2." These values are used to create a "time-weighted average" to create the "A" value. The individual values are listed here for your use:
The middle-of-the-night "A1" sample registered a cortisol value of 16.5ng/mg.
The waking "A2" sample registered a cortisol value of 28.1ng/mg.
These two values are averaged together, taking into account the amount of time each one represents, to create the "A" value of approximately 21.7ng/mg that you will see on the report.
In this particular case, this A2 value is larger than the sample (collected two hours after waking) expected to have the highest cortisol value. Cortisol levels typically rise sharply after waking thanks to the cortisol awakening response. In a case like this where the waking sample (A2) shows higher levels, this cortisol awakening response may have happened while the patient was in bed before rising.



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Organic Acid Tests (OATs)

Ordering Provider:
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Category	Test	Result	Units	Normal Range
Nutritional Organic Acids				
Vitamin B12 Marker (may be deficient if high) - (Urine)				
	Methylmalonate (MMA)	Within range	1.51 ug/mg	0 - 2.5
Vitamin B6 Markers (may be deficient if high) - (Urine)				
	Xanthurenate	Within range	0.74 ug/mg	0.12 - 1.2
	Kynurenate	Within range	2.58 ug/mg	0.8 - 4.5
Biotin Marker (may be deficient if high) - (Urine)				
	b-Hydroxyisovalerate	Within range	8.0 ug/mg	0 - 12.5
Glutathione Marker (may be deficient if low or high) - (Urine)				
	Pyroglutamate	Within range	50.9 ug/mg	28 - 58
Gut Marker (potential gut putrefaction or dysbiosis if high) - (Urine)				
	Indican	Within range	49.2 ug/mg	0 - 100
Neuro-related Markers				
Dopamine Metabolite - (Urine)				
	Homovanillate (HVA)	Low end of range	3.1 ug/mg	3 - 11
Norepinephrine/Epinephrine Metabolite - (Urine)				
	Vanilmandelate (VMA)	Within range	3.4 ug/mg	2.2 - 5.5
Neuroinflammation Marker - (Urine)				
	Quinolate	Within range	6.9 ug/mg	0 - 9.6
Additional Markers				
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
	Melatonin* (Waking)	Within range	42.9 ng/mg	10 - 85
Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)				
	8-OHdG (Waking)	Low end of range	0.65 ng/mg	0 - 5.2

Clinical Support Overview

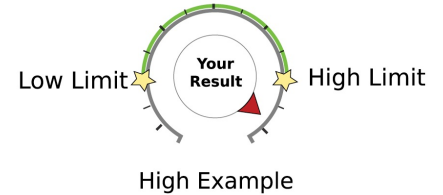
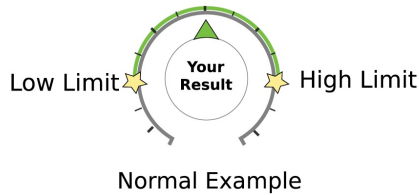
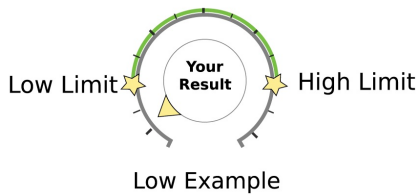
Thank you for choosing DUTCH for your functional endocrinology testing needs! We know you have many options to choose from when it comes to functional endocrinology evaluation, and we strive to offer the best value, the most up-to-date testing parameters and reference ranges, and the greatest clinical support to ensure the most accurate results.

Please take a moment to read through the Clinical Support Overview below. These comments are specific to the patient's lab results. They detail the most recent research pertaining to the hormone metabolites, treatment considerations, and follow-up recommendations. These comments are intended for educational purposes only. Specific treatment should be managed by a healthcare provider. To view the steroid pathway chart, click here [Steroid Pathway Chart](#)

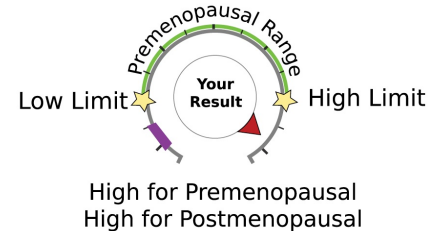
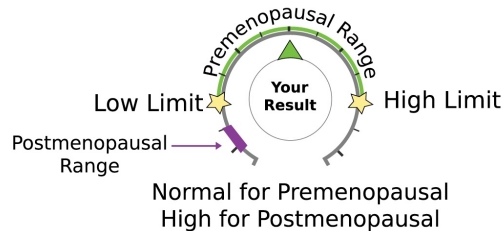
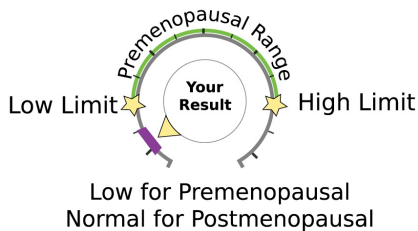
Alert comments:

How to read the DUTCH report

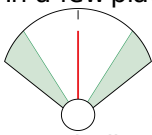
This report is not intended to treat, cure or diagnose any specific diseases. The graphic dutch dials in this report are intended for quick and easy evaluation of which hormones are out of range. Results below the left star are shaded yellow and are below range (left). Results between the stars and shaded 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.



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.



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 irregular menstrual cycles.

According to the data supplied by the patient, the samples were collected on Cycle Day 18. For assessing progesterone, it is ideal that samples are collected around days 19-24 of a 28-day cycle. If samples are collected 10 or more days before the next cycle or closer than three days to the end of a cycle, progesterone value may be lower than they would have been at their peak.

The patient reports symptoms of both estrogen deficiency and excess. Levels of estrogen (and metabolites) should be carefully reviewed.

The patient reported significant symptoms of excess androgen levels.

The patient reported significant fatigue in the afternoon/evening, but not in the morning.

Progesterone Metabolism

Progesterone is made predominately in the ovaries by the corpus luteum following the release of an egg. Progesterone metabolite levels will increase to the premenopausal luteal range (the range established as the green band between the two gold stars) only after the release of an egg. The level of progesterone metabolites seen on the DUTCH test can help determine if ovulation occurred 5-7 days prior to test collection.

The primary role of progesterone is to prepare the endometrium of the uterus for implantation. In addition, it may balance the effects of estrogen, it is a neurosteroid, it acts as a diuretic and raises basal body temperature.

We are measuring metabolites of progesterone 5b-pregnanediol and 5a-pregnanediol. 5b-pregnanediol has less activity in the body but does represent a larger percent of total progesterone metabolism overall. 5a-pregnanediol is often a metabolite of more interest, as it can cross the blood brain barrier and up-regulate GABA activity and is considered neuroprotective to the brain. In some women the 5a-pregnanediol is also the cause of PMDD and irritability due to issues with the GABA receptor's inability to adjust for sensitivity to fluctuating neurosteroids (Dr Briden).

If progesterone levels are in the low or lower end of the luteal reference range compared to estrogen levels, women may experience symptoms such as PMS, menorrhagia, mastalgia, moodiness, anxiety, and/or insomnia.

The metabolites of progesterone are excreted in urine (not the progesterone itself). When ordering the DUTCH Complete and DUTCH Plus reports, you will see a Progesterone Serum Equivalent on the summary page 1. The urine metabolites of progesterone have been proven to correlate strongly to serum progesterone. The Progesterone Serum Equivalent is most accurate with values in the luteal range and becomes more approximate at very low numbers in the postmenopausal range. Cycling women with very high progesterone metabolites may also decrease the accuracy of the serum equivalent calculation. NOTE: If progesterone is taken orally (also with sublingual), these metabolites are elevated from gut metabolism and results do NOT accurately reflect serum levels.

The patient reports use of oral progesterone. The ranges are set for someone on the most common standard dose of 100MG. The ranges given assume the progesterone was taken the day/night of testing and not skipped. 100MG of oral progesterone typically results in urine metabolite values well above the premenopausal reference range (see ranges on first 2 pages of the report), and as a result the ranges are set higher than someone not on progesterone or on progesterone topically. The levels of the b-Pregnanediol correlate with serum progesterone when women are not supplementing. With oral progesterone, the metabolites measured increase to a much greater degree than serum progesterone levels (serum progesterone levels do not raise above 2NG/ML in most studies with accurate measurements). Studies show that the creation of these progesterone metabolites results in significant positive clinical outcomes as the endometrium in the uterus is protected (when estrogen is concurrently given) and sleep disturbances are usually improved (because of large amounts of allo-pregnanolone metabolite having a positive effect on the GABA neurotransmitter to induce relaxation/calming). Be aware that some women respond differently to oral progesterone depending on how much of it gets metabolized down the more biologically active a-pathway (as opposed to the b-pathway).

Serum progesterone levels do not increase much with oral progesterone supplementation because the hormone is so extensively metabolized in the gut. If you desire to increase serum progesterone levels, a different route of administration (ex. vaginal, sublingual) should be considered. It is thought that alpha (a-Pregnanediol) metabolites have more of a clinical effect than beta (b-Pregnanediol) metabolites. Women may need less oral progesterone to achieve help with sleeping if the oral progesterone can push the hormone more down the a-Pregnanediol pathway, however this does not always occur to the degree one might hope. The primary information when on oral progesterone is the relative alpha vs beta metabolism. If the patient did not take their supplement the day of the test, values may be much lower than expected, but will still be above baseline levels for several days.

This [video](#) may assist with understanding results in this situation.

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.

• **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.

For this patient 2-Methoxy-E2 was not reportable due to an interfering substance or instrument irregularity for this compound.

Androgen Metabolism

Androgen Metabolites: DHEA

DHEA and androstenedione are made almost exclusively by the adrenal gland (although a smaller amount is made in the ovaries for). These hormones appear in urine as DHEA-S (DHEA-Sulfate), androsterone and etiocholanolone.

DHEA peaks for men and women in their 20's and 30's, with a slow decline expected with age. DHEA mainly circulates throughout the body as DHEA-s, with interconversion to active DHEA as it reaches various tissues. DHEA is a weak androgen and will predominately convert to androstenedione, which will then convert to testosterone or estrogen. DHEA-s is made by sulfation, has a much longer half-life than DHEA and largely lacks a diurnal rhythm, which is why it is considered the best way to assess DHEA levels in the body. DHEA-s levels can be affected both by the total production as well as by the body's ability to sulfate DHEA.

The best way to assess the total production of DHEA is to add up these three metabolites. As DHEA production decreases quite significantly with age, we provide the age-dependent ranges. Adrenal DHEA serves as the main source of estrogen, progesterone and testosterone for post-menopausal women.

The Total DHEA Production (page 1) was about 942ng/mg which is within the overall range but is below the range for the patient's age-dependent range. This implies that the adrenal glands are not producing appropriate DHEA levels for the patient's age. Low DHEA is associated with depression, diabetes, heart disease, inflammation and immune disorders. It can be caused by hypothyroidism. It can cause fatigue, low mood and low libido. Supplementing DHEA in women often raises both testosterone and estrogen, which may or may not be desirable here. DHEA may increase with adaptogens such as maca and rhodiola, which improve overall adrenal output.

• **Androgen Metabolites: Testosterone**

The DUTCH test measures the total of testosterone glucuronide and testosterone sulfate. These conjugates of testosterone are formed mostly from bioavailable testosterone that undergoes phase 2 metabolism to make it ready for urine excretion. Females make most of their DHEA in the adrenal gland and a fraction of that DHEA trickles down metabolically to testosterone. Testosterone is also made by the ovaries.

Testosterone glucuronide is mostly made by the UGT2B17 enzyme, which also makes the glucuronide forms of 5a-DHT and 5b-androstanediol. Genetic variants of this enzyme reduce the urinary levels of these hormones without affecting serum levels. The genetic variants of UGT2B17 vary in the population from 7-80% (variation dependent on genetic ancestry, with the highest rates in those of Asian descent). Heterozygous individuals show

milder reductions in urinary testosterone than homozygous. For this reason, low and very low levels of urinary testosterone should be confirmed with serum testing before treatment is applied. Serum testing can include free and total testosterone and SHBG.

Testosterone levels may be better understood by also considering its downstream metabolites (5 α -androstane diol, 5 β -androstane diol). 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.

Testosterone levels normally decline with age. Age dependent ranges are provided. Perimenopausal testosterone levels can transiently increase before declining again.

Androgens, specifically DHT and testosterone, help to support skin, connective tissue, bone and muscle integrity and promote dopamine conversion in the brain, which can help with mood and libido.

Testosterone levels for this patient were approximately 10.0ng/mg, which is within range. If the patient complains of androgen imbalance, look at the metabolism and DHEA metabolites for further insight. Also, consider other causes. For example, hair loss, which can be androgenic, can also be caused by hypothyroidism, autoimmune disease, high stress or mineral deficiency. Acne, which can be androgenic, also has dietary triggers for some people, most commonly dairy and sugar.

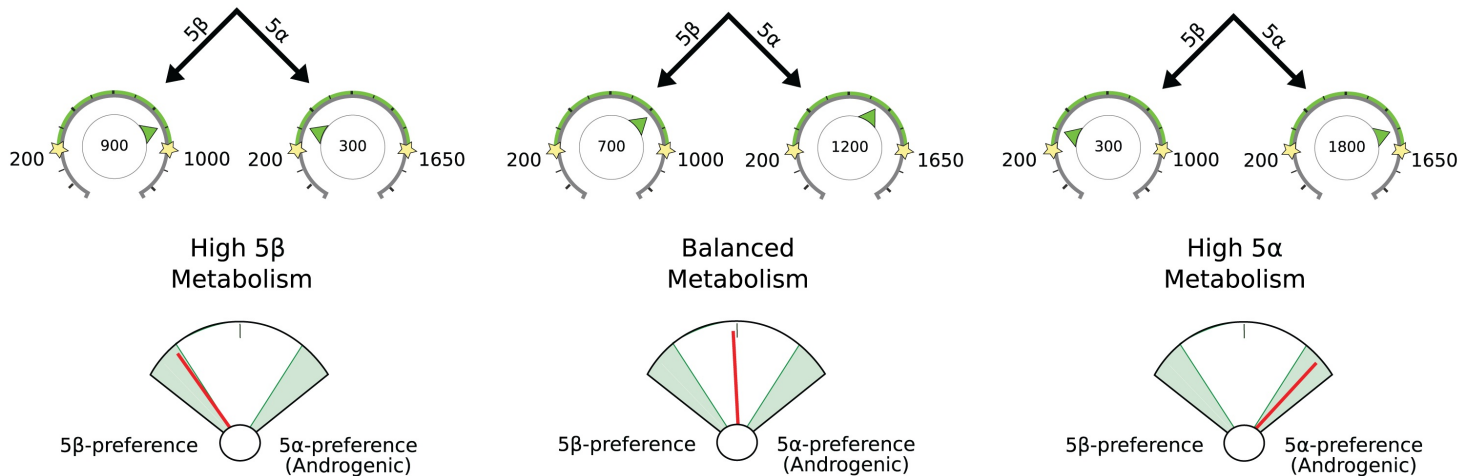
• Androgen Metabolites: 5 α -reductase versus 5 β reductase

5 α -reductase converts testosterone into 5 α -DHT (DHT), which is even more potent (~3x) than testosterone. High levels of DHT can lead to symptoms associated with too much testosterone, including scalp hair loss, hirsutism, acne and oily skin.

Metabolites created down the 5 β -pathway are significantly less androgenic than their 5 α counterparts.

The fan-style gauge below the hormones shows the 5 α or 5 β preference based on etiocholanolone (5 β) and androsterone (5 α) results. The gauge shows the relative ratio of 5 α to 5 β products but does not express the absolute value of DHT or if 5 α -reductase inhibition is or is not indicated. Consider symptoms and look at the 5 α -DHT result if high androgen symptoms are a concern. Progesterone metabolites are also metabolized by 5 α and 5 β enzymes and the balance between its two metabolites can be useful to confirm a 5 α or 5 β preference overall (or tissue specific preference).

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



Neither testosterone or overall levels of DHEA are elevated, but 5 α -metabolism is somewhat preferred over the less androgenic 5 β pathway. In this case, this may be problematic as the patient did note androgen excess symptoms. 5 α metabolism can cause high androgen symptoms due to DHT production but there has to be sufficient levels of androgens to cause high DHT levels, which may or may not be the case for this patient. You may want to explore strategies to block 5 α metabolism (saw palmetto, nettle root, etc.) but you may also want to explore other potential causes (for example, low thyroid can lead to thinning scalp hair).

When assessing androgens in women, 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.

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 when assessing the validity of urinary testosterone testing in an individual patient. If epi-testosterone is much higher than testosterone, serum testosterone assessment should be considered before initiating therapy for low testosterone. Epi-testosterone is suppressed when exogenous testosterone is given, which can serve as a proxy for assessing endogenous testosterone production which can be obscured by the exogenous hormone administration.

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 cortisol is needed in the body, the hypothalamus releases cortisol releasing hormone (CRH) and the pituitary responds by releasing adrenocorticotropic releasing hormone (ACTH), which is the signal to the adrenal gland to release cortisol, DHEA and DHEA-s. It is these adrenal hormones that are assessed on the DUTCH test to understand the patient's HPA axis.

The cortisol awakening response is a complex interaction between the HPA axis and the hippocampus, where ACTH normally surges right after waking leading to the day's highest levels of cortisol. This signal is considered by researchers to be separate from the regular circadian rhythm (the smooth transition from lower cortisol at night to modestly higher cortisol in the morning) and to reflect the person's anticipation of stress during the day, some psychosocial factors such as depression or anxiety and their metabolic state. The waking surge in cortisol helps with energy, focus, morning blood sugar and immune regulation.

As the day progresses, ACTH declines and subsequent cortisol decreases throughout the day, so it is low at night for sleep. This cycle starts over the next morning.

Free cortisol provides negative feedback to CRH & ACTH. When free cortisol is too low, ACTH will surge. ACTH will also surge when a physical or psychological stressor occurs.

Only a small fraction of cortisol is "free" and bioactive. The "free" cortisol is what the person feels in terms of energy and focus. Free cortisol is also what feeds back to the hypothalamus and pituitary gland for ACTH and cortisol regulation. The free cortisol daily pattern is very useful for understanding cortisol and its interaction with the patient's symptoms throughout the day. However, because only a fraction of the cortisol is bioactive, when considering treatments that affect the whole HPA axis, including DHEA, it is essential to measure metabolized cortisol to get a bigger picture.

In urine, we can measure both the total metabolized cortisol (THF) and total metabolized cortisone (THE) excreted throughout the day. These two components better represent the total cortisol production from the adrenal glands than the free cortisol alone. Outside of the HPA axis, metabolism of cortisol occurs with the help of thyroid hormone in the liver. A significant amount of cortisol is also metabolized in adipose tissue.

To best determine total adrenal production of cortisol throughout the day it is important to assess both metabolized cortisol and free cortisol.

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

- **The daily pattern of free cortisol throughout the day, looking for low and high levels:**

Abnormal results should be considered along with related symptoms. Remember that with urine results, the "waking" sample reflects the night's total for free cortisol. The sample collected two hours after waking captures the cortisol awakening response, which is typically the time with the most cortisol secretion.

- **The sum of the free cortisol as an expression of the overall tissue cortisol exposure:**

This total of four free cortisol measurements is the best way to assess the total of free cortisol throughout the day, and this result correlates reasonably well to a true 24-hour urine free cortisol. Do be aware that this measurement does not consider transitory shifts in cortisol in the late morning or early afternoon. This number is calculated from the simple addition of the 4 points, so if a single point is very high or very low, it may skew the number up or down especially if it is the morning "B" point, as it is weighted more heavily in the reference range.

- **The total level of cortisol metabolites:**

We call this calculation "Metabolized Cortisol" which is the sum of a-THF, b-THF and b-THE (the most abundant

cortisol metabolites). While free cortisol is the best assessment for tissue levels of cortisol, it only represents 1-3% of the total produced. The total metabolized cortisol best represents the total glandular output of cortisol for the day, closer to 80% of the total produced.

The patient reports using a glucocorticoid. Please be aware that topical, inhaled, injected and oral steroids (glucocorticoids) can suppress the ACTH signal, resulting in a suppressed adrenal pattern. Please keep this in mind when evaluating these results and contact the lab with any questions or concerns.

While free cortisol levels are on the lower side of normal, these results can be somewhat misleading in this case. Overall cortisol production is best approximated by levels of metabolized cortisol, which are elevated. This implies that overall HPA-Axis activity is elevated. Cortisol clearance is up-regulated in this patient, leaving them with low levels of free cortisol. The patient's cortisol status may be different depending on location within the body. For example, the conversion from the noradrenaline to adrenaline is driven by cortisol and takes place within the adrenal medulla. In this case, this area is likely flooded with high levels of cortisol forcing conversion to adrenaline, whereas the brain (where cortisol has negative feedback on ACTH production) may be more cortisol deficient. Efforts to increase HPA-Axis activity may exacerbate some symptoms. Calming the HPA-Axis, while supporting it in ways that are not excitatory, may be the best course of action.

• **A potential preference for cortisol or cortisone (the inactive form):**

To determine total systemic preference for cortisol or cortisone, it is best to look at which *metabolite* predominates (THF or THE?). This preference can be seen in the fan style gauge. 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. We can see more cortisol or cortisone in difference metabolic conditions. If one is favored over another, read below for potential causes and treatment considerations.

Nutritional Organic Acids

Organic acids are the metabolic byproducts of cellular activity in the body. Organic acid production varies by the individual and can be influenced by foods, environmental toxins, medications or supplements, nutrient status, genetics and more. Organic acids begin to build up when a nutrient cofactor or mineral is not present for a specific reaction to occur. As a response, byproducts (organic acids) build up and can be measured in urine. On the DUTCH test, the organic acids we measure were chosen due to their specific roles in the metabolism and function of enzymes required for hormone and adrenal health and function. As industry standard dictates, the organic acids are measured from the waking sample.

Methylmalonate (MMA)

Methylmalonic acid is a metabolic byproduct of the Citric Acid Cycle (Krebs cycle). Methylmalonic acid requires adenosylcobalamin for conversion to succinyl-CoA and onto ATP synthesis. If someone does not absorb enough B12 from their diet due to low B12-rich food consumption, low stomach acid, has an autoimmune disorder impacting Intrinsic Factor in the gut (required for B12 absorption), or has a MUT enzyme SNP (required for conversion of MMA to Succinyl coA, dependent on adenosylcobalamin) then MMA will build up. Vitamin B12 is required for COMT activity (estrogen methylation, dopamine breakdown) and PNMT activity (the enzyme that takes norepinephrine to epinephrine), but is also critical for memory, energy production (ATP synthesis), gait and more. When MMA is high, consider supporting B12 through foods, digestive support or supplementation.

Xanthurenate & Kynurenate

Xanthurenate and kynurenate are metabolic byproducts in the production of tryptophan to NAD in the liver. If either xanthurenate or kynurenate build up in the urine, it can indicate a need for vitamin B6. This need is amplified if BOTH markers are elevated, and often indicates a more severe deficiency of vitamin B6. Vitamin B6 is critical as a co-factor to over 100 important reactions that occur in the human body and is stored in the highest concentration in muscle tissue.

Tryptophan is converted to NAD by the liver and one of the steps in this pathway requires B6. When B6 is insufficient, xanthurenate is made instead. Xanthurenate can also bind to iron and create a complex that increases DNA oxidative damage resulting in higher 8-OHdG levels. If both the xanthurenate and 8OHdG levels are elevated, there is likely an antioxidant insufficiency.

Kynurenate may also become elevated when patients are B6 deficient because of a different, possibly less B6

dependent pathway. 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 elevations. If levels of estrogen or cortisol are high, it may exacerbate kynurenic acid and increase the need for vitamin B6. As the Xanthurenate and Kynurenate pathways lead to biomarkers with other influence in the body, elevations in these markers may not always agree.

b-Hydroxyisovalerate

b-Hydroxyisovalerate is made when the body is deficient in biotin. This marker has an inverse relationship with biotin, therefore elevated levels represent deficiencies in biotin. Biotin is an important cofactor in mitochondrial function, metabolism of fatty acids, glucose, and protein, as well as ROS production. Biotin deficiency has similar symptoms as other B-vitamin deficiencies but is most often associated with hair loss. Factors that influence biotin levels include inadequate dietary intake, long-term and high-dose B5 supplementation, dysbiosis/gut health, antibiotic use, medications, and biotinidase deficiency.

Pyroglutamate

Pyroglutamate is an intermediate in glutathione recycling and production. Glutathione requires the amino acids cysteine, glycine and glutamate for production. If the body cannot convert pyroglutamate forward to glutathione, it will show up elevated in the urine. High pyroglutamate is an established marker for glutathione deficiency. Remember that glutathione is one of the most potent antioxidants in the human body and 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.

Note: Pyroglutamate in the urine can also be elevated with Italian cheese consumption. Italian Cheeses (parmesan, etc.) may transiently increase pyroglutamate because they use a thermophilic lactobacilli to ripen the cheese- which our gut breaks down into pyroglutamate. This is not clinically significant and only reflects that they ate this style of cheese (if applicable).

Indican

Indican is a byproduct of tryptophan putrefaction by microbes in the gut. Accumulated levels of indican in the urine suggest higher levels of tryptophan putrefaction from gastrointestinal dysbiosis or malabsorption. Production of indican occurs when tryptophan creates indoles in the colon. No other endogenous indoles are metabolized in this way, so when we see indican in the urine, it is directly related to gut production and a direct reflection of gut health. When there is concern of dysbiosis, there may be poor metabolism of sex hormones (including estrogen) along with chronic low-grade inflammation that can impact cortisol production and metabolism. This test is not diagnostic but generally warrants further testing to rule out gut dysbiosis.

Vegetarian and vegan style eating may influence results as these diets have less protein generally, therefore elevated levels are likely stronger suggestions of gut dysbiosis. The amount of indican present does not correlate to the degree of dysbiosis but merely shows that dysbiosis is present. Common causes of high indican include malabsorption of protein as a result of low stomach acid, poor pancreatic function, Celiac disease, the overgrowth of anaerobic bacteria in the colon, small intestinal bacterial overgrowth (SIBO), medications that reduce protein absorption (like proton pump inhibitors or other antacids or H2 blockers), and constipation.

Neuro-related Organic Acids

Neurotransmitters are chemical signals produced by neurons in tissues throughout the body that act as chemical messengers that influence mood, cortisol, heart rate, appetite, muscle contraction, sleep and more. Measuring neurotransmitters directly is difficult because of their instability, and their direct urinary measurements are controversial with respect to how well they reflect the body's level of these neuro-hormones.

Each of the neurotransmitters assessed on the DUTCH test (dopamine, norepinephrine/epinephrine) can be assessed indirectly by measuring their urine metabolites (HVA and VMA respectively). While these metabolites are not a perfect reflection of what is going on in the brain, the scientific literature does affirm their use for a good representation of overall levels of these neurotransmitters in the body.

Homovanillate (HVA)

Homovanillate (HVA) is the primary metabolite of dopamine, a brain and adrenal neurotransmitter that comes from tyrosine (with BH4 and iron as co-factors). Dopamine goes on to create norepinephrine and epinephrine (adrenaline).

Low levels of dopamine are associated with depression, addictions, cravings, apathy, pleasure seeking

behaviors, increased sleepiness, impulsivity, tremors, low motivation fatigue and low mood. High levels of dopamine are associated with agitation, insomnia, mania, hyperactivity, hyper-focus, high stress, anxiety and addictions/cravings/pleasure seeking (to maintain high levels). High HVA can be caused by the use of the following supplements, foods or medications within 72 hours of collecting urine samples: tyrosine, phenylalanine, mucuna, quercetin, bananas, avocados as well as parkinson's medications. If these are being used, the HVA on the DUTCH test may not accurately reflect circulating dopamine levels and should be disregarded.

Vanilmandelate (VMA)

Vanilmandelate (VMA) is the primary metabolite of norepinephrine and epinephrine (adrenaline). The adrenal gland makes cortisol and DHEA (from the adrenal cortex) as well as norepinephrine and epinephrine (from the adrenal medulla). When adrenal hormone output is 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 of catechols) and MAO are needed to make HVA and VMA from dopamine and norepinephrine respectively. If these enzymes are not working properly, HVA and/or VMA may be low in urine, when circulating levels of dopamine and/or norepinephrine/epinephrine may not be low.

Low levels of norepinephrine/epinephrine are associated with addictions, cravings, fatigue, low blood pressure, low muscle tone, intolerance to exercise, depression, and loss of alertness.

High levels of norepinephrine and epinephrine are associated with feelings of stress, aggression, violence, impatience, anxiety, panic, excess worry/hypervigilance, insomnia, paranoia, increasing tingling/burning, loss of memory, pain sensitivity, high blood pressure and heart palpitations.

Quinolate (QA)

Quinolate is a neurotoxin derived from tryptophan. Elevated quinolate is seen in brain and nerve tissue damage, especially in disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron diseases, multiple sclerosis, epilepsy, amyotrophic lateral sclerosis, and major depressive disorder. We can also see elevated quinolate due to low serotonin and need for vitamin B3 (niacin). The causes of elevated quinolate include neuroinflammation, general inflammation, infection, phthalate exposure, and/or oral tryptophan use.

Melatonin (measured as 6-OHMS)

Melatonin is considered one of our sleep hormones. It is made predominately by the pineal gland in response to darkness and is stimulated by melanocyte stimulating hormone (MSH). A low MSH is associated with insomnia and an increased perception of pain. Mold exposure can inhibit MSH as well. The majority of our melatonin production comes from the pineal gland, but melatonin is also made in the gut, and to a lesser extent in the bone marrow, lymphocytes, epithelial cells and mast cells.

The DUTCH test uses the waking (A) sample to test melatonin. The urine sample given on waking reflects overnight hormone production and metabolism. This sample can be used to assess melatonin throughout the night. When patients take a middle of the night urine sample, a large amount of data strongly suggests that the waking sample alone still correlates best to overnight melatonin production, so the waking sample is still used for the DUTCH melatonin result.

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

8-OHdG (8-Hydroxy-2-deoxyguanosine) is a marker for estimating DNA damage due to oxidative stress (from ROS creation). 8-OHdG is considered pro-mutagenic and is a biomarker for various cancer and degenerative disease initiation and promotion states. 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 (to name a few). 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.

Urine Hormone Testing - General Information

What is actually measured in urine? In blood, most hormones are bound to binding proteins. A small fraction of the total hormone levels are "free" and unbound such that they are active hormones. These free hormones are not found readily in urine except for cortisol and cortisone (because they are much more water soluble than, for

example, testosterone). As such, free cortisol and cortisone can be measured in urine and it is this measurement that nearly all urinary cortisol research is based upon. In the DUTCH Adrenal Profile the diurnal patterns of free cortisol and cortisone are measured by LC-MS/MS.

All other hormones measured (cortisol metabolites, DHEA, and all sex hormones) are excreted in urine predominately after the addition of a glucuronide or sulfate group (to increase water solubility for excretion). As an example, Tajic (Natural Sciences, 1968 publication) found that of the testosterone found in urine, 57-80% was testosterone-glucuronide, 14-42% was testosterone-sulfate, and negligible amounts (<1% for most) was free testosterone. The most likely source of free sex hormones in urine is from contamination from hormonal supplements. To eliminate this potential, we remove free hormones from conjugates (our testing can be used even if vaginal hormones have been given). The glucuronides and sulfates are then broken off of the parent hormones, and the measurement is made. These measurements reflect the bioavailable amount of hormone in most cases as it is only the free, nonprotein-bound fraction in blood/tissue that is available for phase II metabolism (glucuronidation and sulfation) and subsequent urine excretion.

Disclaimer: the filter paper used for sample collection is designed for blood collection, so it is technically considered "research only" for urine collection. Its proper use for urine collection has been thoroughly validated.

Reference Range Determination (last updated 07.01.2022)

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 07.01.2022)									
	Low%	High%	Low	High		Low%	High%	Low	High
b-Pregnanediol	20%	90%	600	2000	Cortisol A (waking)	20%	90%	10	50
a-Pregnanediol	20%	90%	200	740	Cortisol B (morning)	20%	90%	30	130
Estrone (E1)	20%	80%	12	26	Cortisol C (~5pm)	20%	90%	7	30
Estradiol (E2)	20%	80%	1.8	4.5	Cortisol D (bed)	0	90%	0	14
Estriol (E3)	20%	80%	5	18	Cortisone A (waking)	20%	90%	40	120
2-OH-E1	20%	80%	5.1	13.1	Cortisone B (morning)	20%	90%	90	230
4-OH-E1	0	80%	0	1.8	Cortisone C (~5pm)	20%	90%	32	110
16-OH-E1	20%	80%	0.7	2.6	Cortisone D (bed)	0	90%	0	55
2-Methoxy-E1	20%	80%	2.5	6.5	Melatonin (6-OHMS)	20%	90%	10	85
2-OH-E2	0	80%	0	1.2	8-OHdG	0	90%	0	5.2
4-OH-E2	0	80%	0	0.5	Methylmalonate	0	90%	0	2.2
DHEA-S	20%	90%	20	750	Xanthurenate	0	90%	0	1.4
Androsterone	20%	80%	200	1650	Kynurenate	0	90%	0	7.3
Etiocolanolone	20%	80%	200	1000	b-Hydroxyisovalerate	0	90%	0	12.5
Testosterone	20%	80%	2.3	14	Pyroglutamate	10%	90%	32	60
5a-DHT	0	80%	0	6.6	Indican	0	90%	0	100
5a-Androstenediol	20%	80%	6	30	Homovanillate	10%	95%	4	13
5b-Androstenediol	20%	80%	20	75	Vanilmandelate	10%	95%	2.4	6.4
Epi-Testosterone	20%	80%	2.3	14	Quinolate	0	90%	0	9.6
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
					Total Estrogens	20%	80%	35	70
					Metabolized Cortisol	20%	90%	2750	6500
					24hr Free Cortisol	20%	90%	65	200
					24hr Free Cortisone	20%	90%	220	450
<p><i>% = 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."</i></p>									