

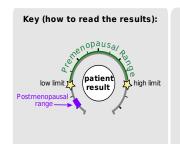
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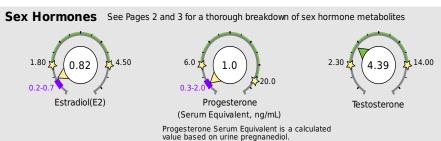
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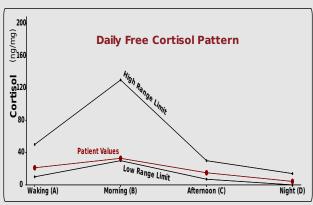
TEST NAME: DUTCH Complete™

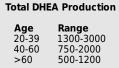
Hormone Testing Summary



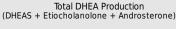


Adrenal Hormones See pages 4 and 5 for a more complete breakdown of adrenal hormones

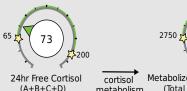








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cortisol metabolism Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

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

PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 7.

- Patient reports being on hormonal birth control. Please see comments section for more information.



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TEST REF: GNL-NL-XXXXX

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TEST NAME: DUTCH Complete™

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Test		Result	Units	Luteal*	Postmenopausal
Progesterone Metabol				Range	Range
b-Pregnanediol	Below luteal range	130.0	ng/mg	600 - 2000	60-200
a-Pregnanediol	Below luteal range	25.9	ng/mg	200 - 740	15-50
Estrogens and Metabo	lites (Urine)				
Estrone(E1)	Below luteal range	6.01	ng/mg	12 - 26	1.0-7.0
Estradiol(E2)	Below luteal range	0.82	ng/mg	1.8 - 4.5	0.2-0.7
Estriol(E3)	Below luteal range	4.5	ng/mg	5 - 18	0.6-4.0
2-OH-E1	Below luteal range	2.23	ng/mg	5.1 - 13.1	0.3-2.0
4-OH-E1	Within luteal range	0.3	ng/mg	0 - 1.8	0-0.3
16-OH-E1	Below luteal range	0.37	ng/mg	0.7 - 2.6	0.2-0.6
2-Methoxy-E1	Below luteal range	1.31	ng/mg	2.5 - 6.5	0.3-1.4
2-OH-E2	Within luteal range	0.35	ng/mg	0 - 1.2	0-0.3
4-OH-E2	Within luteal range	0.13	ng/mg	0 - 0.5	0-0.1
Total Estrogen	Below range	16.0	ng/mg	35 - 70	4.0-15
Metabolite Ratios					
2-OH / 16-OH-E1 Balance	Within range	6.06	ratio	2.69 - 11.83	
2-OH / 4-OH-E1 Balance	Within range	7.49	ratio	5.4 - 12.62	
2-Methoxy / 2-OH Balance	Within range	0.59	ratio	0.39 - 0.67	
Androgens and Metab	olites (Urine)				
DHEA-S	Within range	176.5	ng/mg	20 - 750	
Androsterone	Within range	843.8	ng/mg	200 - 1650	
Etiocholanolone	Within range	828.0	ng/mg	200 - 1000	
Testosterone	Low end of range	4.39	ng/mg	2.3 - 14	
5a-DHT	Within range	2.9	ng/mg	0 - 6.6	
5a-Androstanediol	Within range	19.6	ng/mg	6 - 30	
5b-Androstanediol	Above range	77.4	ng/mg	20 - 75	
Epi-Testosterone	Below range	1.5	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



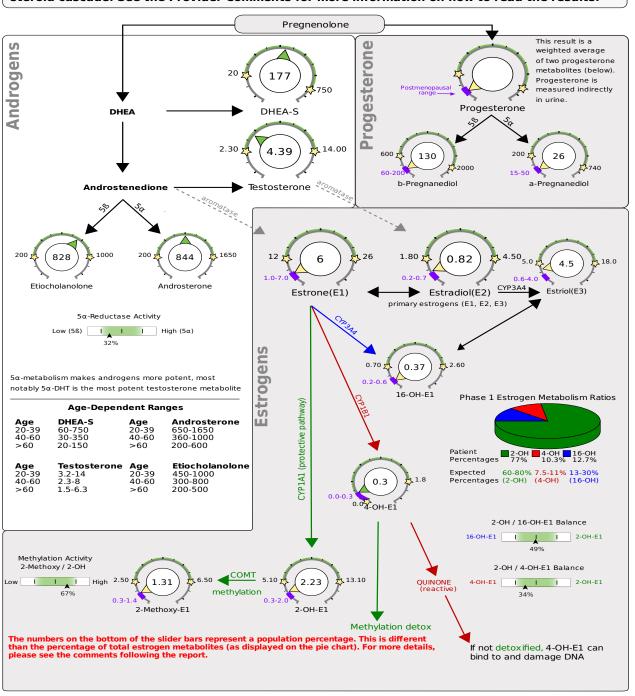
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TEST NAME: DUTCH Complete™

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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TEST NAME: DUTCH Complete™

Category	Test		Result	Units	Normal Range
Creatinine	(Urine)				
	Creatinine A (Waking)	Within range	0.22	mg/ml	0.2 - 2
	Creatinine B (Morning)	Within range	0.49	mg/ml	0.2 - 2
	Creatinine C (Afternoon)	Within range	0.4	mg/ml	0.2 - 2
	Creatinine D (Night)	Within range	0.36	mg/ml	0.2 - 2
Daily Free	Cortisol and Cortisone (Urine)				
	Cortisol A (Waking)	Within range	21.2	ng/mg	10 - 50
	Cortisol B (Morning)	Low end of range	32.9	ng/mg	30 - 130
	Cortisol C (Afternoon)	Within range	15.0	ng/mg	7 - 30
	Cortisol D (Night)	Within range	4.3	ng/mg	0 - 14
	Cortisone A (Waking)	Within range	65.0	ng/mg	40 - 120
	Cortisone B (Morning)	Within range	130.1	ng/mg	90 - 230
	Cortisone C (Afternoon)	Within range	57.3	ng/mg	32 - 110
	Cortisone D (Night)	Within range	27.5	ng/mg	0 - 55
	24hr Free Cortisol	Low end of range	73.4	ng/mg	65 - 200
	24hr Free Cortisone	Within range	279.8	ng/mg	220 - 450
Cortisol Me	etabolites and DHEA-S (Urine)				
	a-Tetrahydrocortisol (a-THF)	Within range	181.9	ng/mg	75 - 370
	b-Tetrahydrocortisol (b-THF)	Within range	1971.8	ng/mg	1050 - 2500
	b-Tetrahydrocortisone (b-THE)	Within range	2599.5	ng/mg	1550 - 3800
	Metabolized Cortisol (THF+THE)	Within range	4753.2	ng/mg	2750 - 6500
	DHEA-S	Within range	176.5	ng/mg	20 - 750



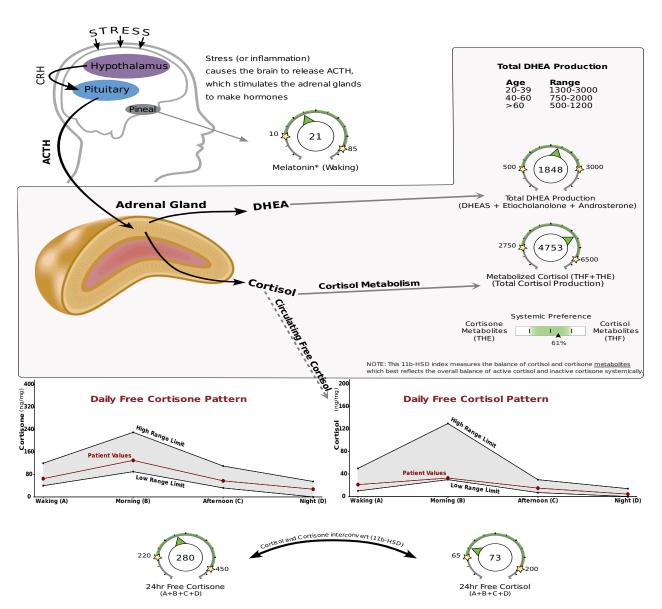
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TEST NAME: DUTCH Complete™



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 5.5ng/mg.

The waking "A2" sample registered a cortisol value of 38.8ng/mg.

These two values are averaged together, taking into account the amount of time each one represents, to create the "A" value of approximately 21.2ng/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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TEST NAME: DUTCH Complete™

Category	Test		Result	Units	Normal Range					
	N	utritional Organic Aci	ds							
Vitamin B12	Marker (may be deficient if high	h) - (Urine)								
	Methylmalonate (MMA)	Within range	2.1	ug/mg	0 - 2.5					
Vitamin B6 M	arkers (may be deficient if high	n) - (Urine)								
	Xanthurenate	Within range	1.09	ug/mg	0.12 - 1.2					
	Kynurenate	Above range	5.0	ug/mg	0.8 - 4.5					
Biotin Marker	(may be deficient if high) - (Ur	rine)								
	b-Hydroxyisovalerate	Within range	7.5	ug/mg	0 - 12.5					
Glutathione N	Narker (may be deficient if low	or high) - (Urine)								
	Pyroglutamate	Below range	27.0	ug/mg	28 - 58					
Gut Marker (p	potential gut putrefaction or dy	sbiosis if high) - (Urine)								
	Indican	Within range	71.8	ug/mg	0 - 100					
	Neuro-related Markers									
Dopamine Me	etabolite - (Urine)									
	Homovanillate (HVA)	Within range	5.2	ug/mg	3 - 11					
Norepinephri	ne/Epinephrine Metabolite - (Ui	rine)								
	Vanilmandelate (VMA)	Within range	3.1	ug/mg	2.2 - 5.5					
Neuroinflamn	nation Marker - (Urine)									
	Quinolinate	Within range	7.3	ug/mg	0 - 9.6					
		Additional Markers								
Melatonin (*n	neasured as 6-OH-Melatonin-S	Sulfate) - (Urine)								
	Melatonin* (Waking)	Low end of range	21.0	ng/mg	10 - 85					
Oxidative Str	ess / DNA Damage, measured			-						
	8-OHdG (Waking)	Within range	3.8	ng/mg	0 - 5.2					



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TEST NAME: DUTCH Complete™

Clinical Support Overview

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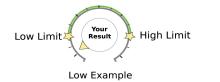
TEST NAME: DUTCH Complete™

How to read the DUTCH report

This report is not intended to treat, cure or diagnose any specific diseases.

DUTCH Dials

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.







Normal Example

High Example

DUTCH Slider Bars

In a few places on the graphic pages, you will see slider bars. For adrenal hormones, you will see one to represent the balance between cortisol and cortisone metabolites. These bars indicate the relative ratio of the metabolites noted. The percentage stated is a population percentage, and so a result of 50%, as in this example (with the slider arrow in the middle of the bar) indicates that the ratio is higher than 50% of individuals tested, or right in the middle of the population's range. If the ratio between the metabolites is "low", the arrow will slide to the left and represent a smaller percentage 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 no menstrual cycles.

The patient reported significant symptoms of excess androgen levels.

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, mastaglia, moodiness, anxiety, and/or insomnia.

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TEST NAME: DUTCH Complete™

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 reported using a hormonal birth control (BC) method (pill, ring, patch or implant) which works by blocking brain signals to the ovaries and subsequently, ovarian hormone production. Endogenous estrogen and progesterone are expected to be low on the DUTCH test when this type of hormonal contraception is used and these low hormones can indicate that the hormonal contraception is likely working. Note that oral BC pills increase SHBG and thus may result in lower testosterone on this test. If the patient would like to know her hormone levels without the influence of BC, it is suggested she wait 3 full menstrual cycles after stopping her BC before retesting, and she should use non-hormonal forms of BC during that time if she wishes to avoid pregnancy. Please note this comment will appear for up to 3 months after a patient reports stopping birth control. Although the hormones in the pill are cleared out of the system within 1 week after discontinuing, some find their ovarian hormone production is not at baseline for several months.

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.

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TEST NAME: DUTCH Complete™

Androgen Metabolism

Androgen Metabolites: DHEA

DHEA and androstenedione are made almost exclusively by the adrenal glands. 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. Adrenals serve as the main source of estrogen, progesterone and testosterone for post-menopausal women.

The Total DHEA Production (page 1) was about 1,848ng/mg which is within the overall range and also within the age-dependent range for this patient. This implies that the adrenal glands are producing appropriate DHEA levels.

• 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 (5aandrostanediol, 5bandrostanediol). 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.

Epi-testosterone (epi-T) is made at about the same rate as testosterone but is not androgenic. In cases where testosterone in urine is low, such as with the UGT2B17 deletion discussed above, epi-T may be used as a proxy for testosterone production, meaning that higher epi-T levels may indicate that a low testosterone result is falsely low. After menopause, epi-T production is less reliable as a marker of testosterone production as epi-T levels drop more sharply than does testosterone during the menopause transition. While epi-T may have limited utility in some cases, it does enhance the picture when taking androgen metabolites together as a whole. 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.

The testosterone result 4.39ng/mg is in range for the patient's age. Review the levels of all androgens, androgenic metabolism, and patient symptoms for a complete assessment.

• Androgen Metabolites: 5a-reductase versus 5b reductase

5a-reductase converts testosterone into 5a-DHT (DHT), which is even more potent (\sim 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 5b-pathway are significantly less androgenic than their 5a counterparts.

The slider bars below the hormones show the 5a or 5b preference based on etiocholanolone (5b) and

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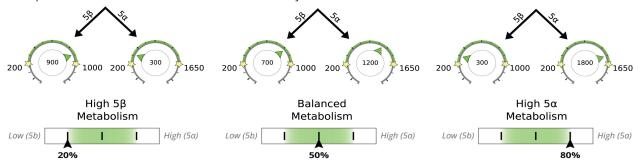
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TEST NAME: DUTCH Complete™

androsterone (5a) results. The slider shows the relative ratio of 5a to 5b products but does not express the absolute value of DHT or if 5a-reductase inhibition is or is not indicated. Consider symptoms and look at the 5a-DHT result if high androgen symptoms are a concern. 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 overall (or tissue specific preference).

Example of how to read sliders for 5a-reductase activity:



Neither testosterone or overall levels of DHEA are elevated, and 5a-metabolism is not elevated. However, the patient does complain of some high androgen symptoms. If the primary complaint is thinning scalp hair, it is not likely that DHT production is to blame. Other causes of these symptoms (thyroid, cortisol, iron...etc) should be explored.

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 considered before initiated 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

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COLLECTED: 00-XXX-2024 RECEIVED: 00-XXX-2024 TESTED: 00-XXX-2024

TEST REF: GNL-NL-XXXXX XXXXXXXXXXX

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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.

Free cortisol levels are on the lower side of the reference range. Levels of metabolized cortisol confirm that overall cortisol production is reasonable, and the actual diurnal pattern of free cortisol should be examined to further examine cortisol production.

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 slider bar. 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 different metabolic conditions. For example, a preference for cortisol indicates possible inflammation, insulin resistence or hypothyroidism. A preference for cortisone can indicate chronic stress or chronic infection (such as the later stages of a virus or common cold).

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 an MUT enzyme SNP (required for

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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 80hdG 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.

The kynurenate level is high. This can indicate B6 deficiency, chronic stress, oxidative stress, inflammation, and possibly LPS bacteria in the gut. Production of kynurenate from tryptophan is upregulated with inflammation, high estrogen, and high cortisol. High kynurenate has been found in schizophrenia, Alzheimer's, cluster headaches, chronic migraines, and psychosis. Certain foods, supplements, and diets can cause high kynurenate without indicating a health issue such as ketogenic diets, and diets containing bee products such as honey or propolis, white potatoes, raw broccoli, basil, and thyme. Because kynurenate is a metabolite of tryptophan metabolism, supplementing tryptophan within 72 hours of collecting DUTCH samples can cause elevated urinary kynurenate without indicating any of the issues listed above. Please keep supplements and foods in mind when interpreting results.

With high kynurenate, consider support of this pathway with vitamin B6, especially if xanthurenate is also elevated, in addition to antioxidants including vitamin C, vitamin E, ALA, magnesium and taurine. Other support includes considering huperzine A or Galantamine if neurological symptoms are present.

b-Hydroxyisovalerate

b-Hydroxyisovalerate is made when the body is deficient in biotin. Elevated levels may indicate biotin deficiency. Biotin is an important cofactor in mitochondrial function, metabolism of fatty acids, glucose, and protein, and ROS production. Biotin deficiency has similar symptoms as other B-vitamin deficiencies but is most often associated with hair loss. Factors influencing biotin levels include inadequate dietary intake, long-term and highdose B5 supplementation, dysbiosis/gut health, antibiotic use, medications, and biotinidase deficiency. Note: If beta-hydroxy-beta-methylbutyrate (HMB), an amino acid conjugate supplement, is taken within 72 hours of testing, we can see high levels of b-hydroxyisovalerate without indicating a biotin deficiency. Please check with supplements when interpreting results.

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).

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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. Chronic dysbiosis can impact sex hormone metabolism, cause inflammation, and influence cortisol levels and metabolism. High urinary indican suggests further testing to rule out gut dysbiosis.

Vegetarian and vegan diets have less protein, therefore elevated levels with these diets 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 because 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. Urinary indican can increase with recent (<72 hours) tryptophan supplementation without indicating dysbiosis. Please keep supplements in mind when interpreting the result.

Neuro-related Markers

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.

Quinolinate (QA)

The quinolinate is high. Quinolinate is a neurotoxin derived from tryptophan metabolism in the context of high

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inflammation or high cortisol. Elevated quinolinate has been 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 quinolinate due to low serotonin and need for vitamin B3 (niacin). If tryptophan supplements are taken within 72 hours of collecting DUTCH samples, there may be high levels of quinolinate in the urine which may not be associated with neuroinflammation. Keep supplements in mind when interpreting results.

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.

Please note that some foods contain small amounts of melatonin that are unlikely to increase circulating levels of melatonin, but may increase metabolites in urine due to first pass metabolism. The most significant of these foods are tomatoes, walnuts, strawberries and caffeinated coffee. These foods are thought to contribute to mildly elevated urinary melatonin. Extremely high urinary melatonin is seen when melatonin is supplemented directly. This is also due to first pass metabolism and is not an accurate reflection of circulating melatonin.

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.

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Reference Range Determination (last updated 5.1.2024)

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 Ref	ference Ra	inges (Updated 05.1.2024)				
	Low%	High%	Low	High		Low%	High%	Low	High
				,	SUBSTRUCTION S		,		
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	2.5
2-16-ratio	20%	80%	2.69	11.83	Xanthurenate	0	90%	0.12	1.2
2-4-ratio	20%	80%	5.4	12.62	Kynurenate	0	90%	0.8	4.5
2Me-2OH-ratio	20%	80%	0.39	0.67	b-Hydroxyisovalerate	0	90%	0	12.5
DHEA-S	20%	90%	20	750	Pyroglutamate	10%	90%	28	58
Androsterone	20%	80%	200	1650	Indican	0	90%	0	100
Etiocholanolone	20%	80%	200	1000	Homovanillate	10%	95%	3	11
Testosterone	20%	80%	2.3	14	Vanilmandelate	10%	95%	2.2	5.5
5a-DHT	0	80%	0	6.6	Quinolinate	0	90%	0	9.6
5a-Androstanediol	20%	80%	6	30	29 3				
5b-Androstanediol	20%	80%	20	75	Calculated Values	50 00			
Epi-Testosterone	20%	80%	2.3	14	Total DHEA Production	20%	80%	500	3000
a-THF	20%	90%	75	370	Total Estrogens	20%	80%	35	70
b-THF	20%	90%	1050	2500	Metabolized Cortisol	20%	90%	2750	6500
b-THE	20%	90%	1550	3800	24hr Free Cortisol	20%	90%	65	200
					24hr Free Cortisone	20%	90%	220	450

% = 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."

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