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INTERPRETIVE GUIDE

Second Edition

Published February 2025

PRECISION ANALYTICAL INC.
CREATORS OF THE DUTCHTEST®

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— INTERPRETIVE GUIDE —

By Precision Analytical Inc.

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DUTCH Interpretive Guide

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TABLE OF CONTENTS

01 | Introduction to DUTCH 10–42

WELCOME 11
ANATOMY OF A DUTCH TEST 12
- How to Read a DUTCH Test 12

REFERENCE RANGE DETERMINATION 14
- Overview 14
- Clinical Utility 15
- How DUTCH is Different 15

DUTCH BIOMARKER KEY 17
CAVEATS 21
- To Test or Not to Test? 21
- Birth Control Method 21
- Physical State 22
- UGT Deletion 23
- Additional Scenarios 24

WHEN TO COLLECT 25
- Cycling Females 25
- Postmenopausal Females 26
- Males 26
- Ovulation Predictor Kits (OPKs) 26
- Basal Body Temperature (BBT) 27

MONITORING (B)HRT WITH DUTCH 28
- Overview 28
- Estrogen Therapy 28
- Progesterone Therapy 31
- Pregnenolone Therapy 33
- Testosterone Therapy 34
- DHEA Therapy 35

THE DUTCH STEROID PATHWAY 39
- Overview 39
- How to Read the Chart 39
- Contributing Factors 39

02 | Sex Hormones: Cycling Female 42–64

THE MENSTRUAL CYCLE 43

Please Note:

The information in this handout is provided for informational and educational purposes only and is not medical or treatment advice. Any information and statements regarding dietary or herbal supplements have not been evaluated by the Food and Drug Administration and are not intended to diagnose, treat, cure, or prevent any disease. The use of any information provided in this handout is solely at your own risk.

ESTROGEN 44
Overview 44
Estradiol is Low 44
Estradiol is Elevated 45
Estradiol is Within Range 45
ESTROGEN DOMINANCE PATTERNS 46
Overview 46
Pattern Examples 46
PROGESTERONE 50
Overview 50
Progesterone is Low 50
Progesterone is Elevated 51
Progesterone is Within Range 51
Oral Progesterone Reference Ranges 52
PROGESTERONE METABOLISM 53
Overview 53
Clinical Significance 53
TESTOSTERONE 54
Overview 54
Testosterone is Low 55
Testosterone is Elevated 55
Testosterone is Within Range 56
DHEA 57
Overview 57
DHEA is Low 57
DHEA is Elevated 58
ANDROGEN METABOLISM 59
Overview of 5-alpha vs 5-beta 59
Clinical Significance 59
ANNOTATIONS 61

03 | Sex Hormones: Postmenopausal Female 64–94

INTRODUCTION 65
ESTROGEN 66
Overview 66
Estradiol is Low 66
Estradiol is Elevated 67
Estradiol is Within Range 67
ESTROGEN DOMINANCE PATTERNS 68
Overview 68
Pattern Examples 68
PROGESTERONE 71
Overview 71
Progesterone is Low 72
Progesterone is Elevated 72
Progesterone is Within Range 72
Oral Progesterone Reference Ranges 73
PROGESTERONE METABOLISM 74
Overview 74
Clinical Significance 74
TESTOSTERONE 75
Overview 75
Testosterone is Low 76
Testosterone is Elevated 76
Testosterone is Within Range 77
DHEA 78
Overview 78
DHEA is Low 78
DHEA is Elevated 79
ANDROGEN METABOLISM 80
Overview of 5-alpha vs 5-beta 80
Clinical Significance 81
ANNOTATIONS 82

04 | Sex Hormones: Male 94–98

INTRODUCTION 85
TESTOSTERONE 86
Overview 86
Testosterone is Low 87
Testosterone is Elevated 87
Testosterone is Within Range 88
DHEA 89
Overview 89
DHEA is Low 89
DHEA is Elevated 90
ANDROGEN METABOLISM 91
Overview of 5-alpha vs 5-beta 91
Clinical Significance 92
ESTROGEN 93
Overview 93
Estradiol is Low 93
Estradiol is Elevated 94

PROGESTERONE 95
Overview 95
Progesterone is Low 95
Progesterone is Elevated 96
ANNOTATIONS 97

05 | Estrogen Detoxification 98–106

INTRODUCTION 99
PHASE 1 DETOX 100
Overview 100
2-Hydroxy-Estrone (2-OH-E1) 100
4-Hydroxy-Estrone (4-OH-E1) 100
16-Hydroxy-Estrone (16-OH-E1) 101
PHASE 2 DETOX 102
Overview 102
2-Methoxy-Estrone (2-Me-E1) 102
PHASE 3 DETOX 104
Overview 104
ANNOTATIONS 105

06 | Cortisol 106–124

INTRODUCTION 107
EVALUATING CORTISOL 108
Overview 108
Using the DUTCH Test 108
Signs & Symptoms of Low Cortisol 109
Signs & Symptoms of Elevated Cortisol 109
TOTAL FREE CORTISOL 110
Overview 110
Signs, Symptoms, & Associated Conditions 110
Special Cases 111
THE CORTISONE SHADOW 112
Overview 112
How to Read the Free Cortisone Pattern 113
Pattern Examples 113
Cortisol (Hydrocortisone Cream) Contamination 114
METABOLIZED CORTISOL 115
Overview 115
Signs, Symptoms, & Associated Conditions 115
CORTISOL CLEARANCE RATE 116
Overview 116
CCR is Slow: Metabolized Cortisol < Free Cortisol 116
CCR is Fast: Metabolized Cortisol > Free Cortisol 116
CCR is Normal: Metabolized Cortisol = Free Cortisol 117
THE VS THF 118
Estimating Systemic Preference 118
THE Preference 118
THF Preference 118
CORTISOL AWAKENING RESPONSE 119
Overview 119
Measuring the CAR 119
CAR is Absent 120
CAR is Low 120
CAR is Elevated 121
CAR is Within Range 121
ANNOTATIONS 122

07 | Organic Acids 124–136

INTRODUCTION 125
NUTRITIONAL MARKERS 126
Methylmalonate (MMA) 126
Xanthurenate & Kynurenate 126
Pyroglutamate 127
b-Hydroxyisovalerate 128
Indican 129
NEURO-RELATED MARKERS 130
Homovanillate (HVA) & Vanilmandelate (VMA) 130
Quinolinate 131
ADDITIONAL MARKERS 133
6-OH-Melatonin Sulfate 133
8-Hydroxy-2-Deoxyguanosine (8-OHdG) 134
ANNOTATIONS 135

08 | References 136–172

APPENDICES 138
Appendix A: DUTCH Test Patterns 138
Appendix B: Potential Support Considerations 139
Appendix C: DUTCH Test Sample Report 143
Appendix D: DUTCH Test Reference Ranges 149
GLOSSARY 153
BIBLIOGRAPHY 157
LIST OF CONTRIBUTORS 159

SECTION ONE

Introduction to DUTCH

Welcome

Thank you for choosing the DUTCH Test™ for your functional endocrinology testing needs. We know you have many options to choose from when it comes to functional endocrinology evaluation, and so we strive to offer the best value, the most scientifically relevant testing parameters and reference ranges, and the greatest clinical and educational support.

The DUTCH Interpretive Guide was written with you, our providers, in mind. DUTCH stands for dried urine test for comprehensive hormones. We included the word “comprehensive” to emphasize that the DUTCH Test provides a more complete picture of a person’s hormone health than saliva, 24-hour urine and serum testing. We also understand that this comprehensiveness can add complexity to test interpretation. To better support our providers in their ability to interpret the DUTCH Test, we have organized all the information that is often discussed during our clinical consults into this handbook. It is our hope that you can use this guide not only to become an expert at DUTCH Test interpretation, but also to acquire a deeper, more extensive understanding of female and male hormone health overall.

This guide is intended to be used in combination with the DUTCH Treatment Guide, the DUTCH Mini Guides, and other DUTCH educational resources. We recommend using the DUTCH Interpretive Guide first to interpret the DUTCH Test results, and then the DUTCH Mini Guides and DUTCH Treatment Guide to evaluate support considerations.

LET’S GET STARTED ▸▸

ANATOMY OF A DUTCH TEST

HOW TO READ A DUTCH TEST

The DUTCH Test uses dials and sliders to visually present the test results. They have been designed to allow for quick and easy evaluation of which hormones are out of range, see **Figure 1.1**.

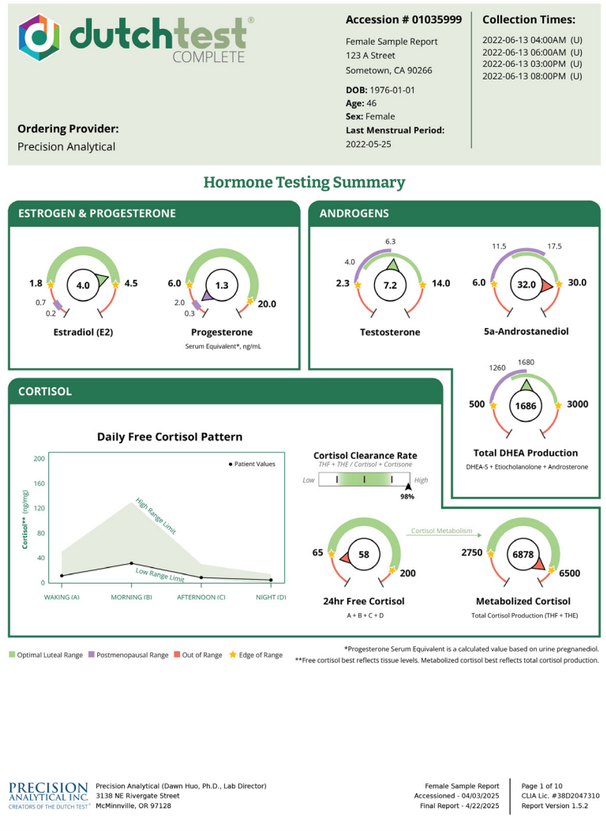


Figure 1.1: ***Page 1 of an Example DUTCH Complete Report***
The DUTCH Test has been visually designed to allow for quick and easy evaluation of which hormones are out of range.

Estrogen & Progesterone Dials on a DUTCH Female Report

The sex hormone dials on the DUTCH female report have two reference ranges, see **Figure 1.2.** On female estrogen and progesterone dials, the green shaded area represents the reference range for the luteal phase of the menstrual cycle. On female androgen dials, the green shaded area represents the premenopausal reference range. For all female sex hormone dials, the purple shaded area represents the postmenopausal reference range.

Androgen Dials on a DUTCH Male Report

The androgen dials on the DUTCH male report have two reference ranges, see **Figure 1.3**. The light green shaded area represents the range for males age 18-40 years, while the dark green shaded area represents the range for males age 41-60+ years.

Regarding all sex hormone dials, these dials represent reference ranges for females 20 years and older and males 18 years and older. Therefore, use caution if interpreting a person's DUTCH Test using these ranges if the female is younger than 20 or if the male is younger than 18.

Androgens naturally decline with age, so referencing the age-dependent ranges offers a better representation of expected levels when interpreting the androgen dials, see **"Reference Range Determination" on page 14** for more information.

All Other DUTCH Dials

For male estrogen dials and male and female cortisol dials, the arrows on DUTCH dials, see **Figure 1.4**, are shaded according to their position within the established reference ranges:

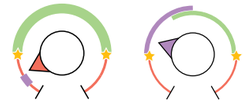
- Results below the left star are shaded yellow and are considered below the reference range.
- Results between the stars are shaded green and are considered within the reference range.
- Results beyond the star on the right and shaded red are considered above the reference range.

Population Sliders

Population sliders on the DUTCH Test visually present the patient's result as a population percentile and provide a visualization of their metabolic preference. The population percentile is plotted on the slider low to high, from left to right, evenly across the slider. The patient's result is shown with a black arrow and their percentile is listed. For example, in **Figure 1.4**. we see the 2OH/4OH ratio is at the 97th percentile. This means that the patient's result is higher than 96% of the population. Since the 2-OH is in the numerator of the ratio, a high result indicates 2-OH is very dominant for this patient.

Figure 1.2: Sex Hormone Dials on a DUTCH Female Report

- Low/high limit of the luteal range
- Luteal range
- Postmenopausal range

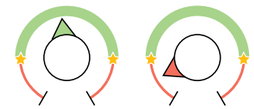


On the female estrogen and progesterone dials, the green shaded area represents the reference range for the luteal phase of the menstrual cycle. On the female androgen dials, the green shaded area represents the premenopausal reference range. For all sex female hormone dials, the purple shaded area represents the postmenopausal reference range.

Figure 1.3: Androgen Dials on a DUTCH Male Report

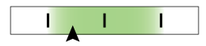
Figure 1.4: All Other DUTCH Dials

- Low/high limit of the normal range
- Within range
- Above the reference range



The arrows on all androgen, cortisol, and male dials are shaded according to their position within the established reference ranges.

Figure 1.5: Population Slider



Population sliders on the DUTCH Test visually present the patient's result as a population percentile and provide a visualization of their metabolic preference.

REFERENCE RANGE DETERMINATION

OVERVIEW

We aim to make the reference ranges for our 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 female'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 females on birth control or with any conditions that may be related to estrogen production.

Regarding all sex hormone dials, these dials represent reference ranges for females 20 years and older and males 18 years and older. Therefore, use caution if interpreting a person's DUTCH Test using these ranges if the female is younger than 20 or if the male is younger than 18.

Androgens naturally decline with age, so referencing the age-dependent ranges offers a better representation of expected levels when interpreting the androgen dials, as seen in **Figure 1.6** and **Figure 1.8**.

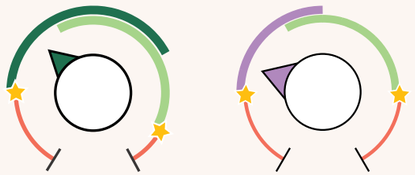


Figure 1.8: ***Example T dials***
The androgen dials on the DUTCH report have two reference ranges. On male androgen dials (left example above), the light green shaded area represents the range for males age 18-40 years, while the dark green shaded area represents the range for males age 41-60+ years. On female androgen dials (right example above), the green shaded area represents the premenopausal reference range, while the purple shaded area represents the postmenopausal reference range.

Figure 1.6: Female androgen reference ranges

Age-Dependent Ranges	
Age	**DHEA-S**
20-39	150-1500
40-60	60-800
>60	30-300

Etiocholanolone		**Androsterone**	
20-39	800-1500	20-39	1500-3000
40-60	600-1200	40-60	1000-2000
>60	400-1000	>60	500-1000

5ß-androstanediol		**5α-androstanediol**	
20-39	70-250	20-39	60-250
40-60	55-210	40-60	50-180
>60	40-150	>60	30-130

Testosterone		**5α-DHT**	
18-25	35-115	20-39	9-25
26-40	30-95	40-60	7-20
41-60	25-80	>60	5-16
>60	20-60		

Androgens naturally decline with age. The male and female androgen dials include age-dependent ranges.

Figure 1.7: Male androgen reference ranges

Age-Dependent Ranges	
Age	**DHEA-S**
20-39	150-1500
40-60	60-800
>60	30-300

Etiocholanolone		**Androsterone**	
20-39	800-1500	20-39	1500-3000
40-60	600-1200	40-60	1000-2000
>60	400-1000	>60	500-1000

5ß-androstanediol		**5α-androstanediol**	
20-39	70-250	20-39	60-250
40-60	55-210	40-60	50-180
>60	40-150	>60	30-130

Testosterone		**5α-DHT**	
18-25	35-115	20-39	9-25
26-40	30-95	40-60	7-20
41-60	25-80	>60	5-16
>60	20-60		

Androgens naturally decline with age. The male and female androgen dials include age-dependent ranges.

CLINICAL UTILITY

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 males and females. Modestly elevated testosterone is associated with unwanted symptoms in females more so than in men, so the high range limit is set at the 80th percentile in females and the 90th percentile for men.

NOTE

The 90th percentile is defined as a result higher than 90% 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.

HOW DUTCH IS DIFFERENT

For estrogen and progesterone metabolites, our reference ranges don't overlap. This means you can easily determine whether your patients are in the postmenopausal or luteal ranges, or somewhere in between.

"Mind the Gap"

With a significant gap between estrogen sufficiency and deficiency, you'll be able to accurately place your patient between the postmenopausal and luteal ranges in "the gap". This gap doesn't exist in available saliva testing and is an important piece of information that can help you prescribe and treat effectively.

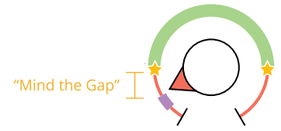
DUTCH Methodology

With an LC/MS assay, the DUTCH Test can more accurately detect nano-level hormone concentrations that show a distinct gap between postmenopausal and luteal reference ranges, improving your ability to precisely characterize your patient's estrogen status. This amount of detail is overlooked by saliva labs and can add more clarity to a patient's hormone status, see **Figure 1.9**.

Clinical Application

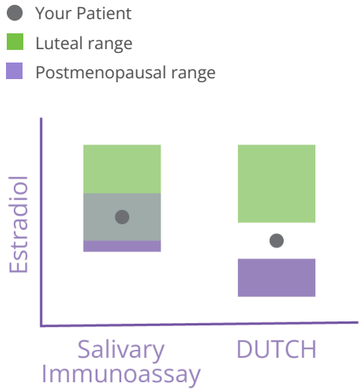
The goal of hormone replacement therapy (HRT) is often to push a woman's estradiol levels out of the postmenopausal (PMP) range and offer her relief from uncomfortable and troublesome symptoms. This is difficult to accomplish when PMP and luteal levels seem to exist in the same space for many of the most commonly used lab tests. Without meaningful differentiation between these two reference ranges, HRT dosing becomes difficult.

Figure 1.9: DUTCH Test Androgen Dials



The female estrogen and progesterone dials show a distinct gap between postmenopausal and luteal reference ranges, improving your ability to precisely characterize your patient's estrogen status.

Figure 1.10: DUTCH Methodology



DUTCH Testing may improve treatment protocols that involve estrogen therapy because DUTCH uses a highly sensitive LC/MS assay which allows for the clear differentiation between the postmenopausal & luteal reference ranges.

In the graph, see **Figure 1.10**, you can see the clear separation between the reference ranges for luteal and postmenopausal estradiol concentration. We refer to this space between ranges as "the gap", and its clinical significance is measurable. Providers should "mind the gap" and pay close attention to those patients whose results fall between PMP and luteal reference ranges.

Let's dive into one of those difficult patient presentations. Imagine a 45-year-old female patient approaches you with symptoms like fatigue, hot flashes, night-sweats, and brain fog. She's interested in HRT to alleviate her symptoms.

The way you proceed with your diagnosis and treatment plan is critical to your patient's well-being and the health of your practice. If you order the standard salivary immunoassay, you might find that her estradiol results are within the expected range for a postmenopausal female and also for a premenopausal woman. What do you do? Many of these symptoms can have causes other than estrogen deficiency.

Prescribing the right HRT dosage (if at all) is nearly impossible in this situation. Without meaningful differentiation between these two ranges (postmenopausal and luteal) you run the risk of misdosing your patient. It's difficult to move forward confidently with treatment plans when you can't get definite results from your hormone test.

DUTCH providers have an edge over other clinicians. With superior sensitivity, DUTCH clarifies the blurred lines between menopausal and luteal estradiol reference ranges that other tests can't define, see **Figure 1.11**. [a]

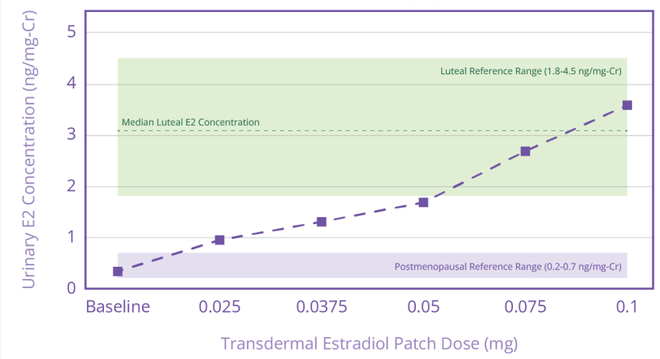


Figure 1.11: ***Monitoring Estradiol (E2) Patch Therapy***
The clear differentiation between the luteal and postmenopausal reference ranges on the DUTCH Test allows for the precise measurement of E2 in women using various patch doses and helps to correlate findings with clinical outcomes.

[a] Mark Newman, MS; Doreen Saltiel, MD, JD; Bryan P. Mayfield, PharmD; Frank Stanczyk, PhD. "Monitoring Estradiol Patch Therapy with a Validated Dried Urine Assay." Paper presented at NAMS, 2021. Washington, DC.

DUTCH BIOMARKER KEY

a-Pregnanediol

Progesterone metabolite that modulates gamma-aminobutyric acid (GABA) receptors in the central nervous system (CNS) and can lead to improvements in mood and sleep.

b-Pregnanediol

Most prominent progesterone metabolite, however, doesn't modulate GABA receptors.

Estrone (E1)

Moderate strength estrogen.

Estradiol (E2)

Most potent, biologically active estrogen.

Estriol (E3) / 16-Hydroxy-Estradiol (16-OH-E2)

Least potent, weak estrogen derived from estradiol and 16-OH-E1.

2-Hydroxy-Estrone (2-OH-E1)

Phase 1 estrone (E1) metabolite; 2-OH metabolites are the most stable phase 1 metabolites.

2-Hydroxy-Estradiol (2-OH-E2)

Phase 1 estradiol (E2) metabolite; 2-OH metabolites are the most stable phase 1 metabolites.

4-Hydroxy-Estrone (4-OH-E1)

Phase 1 estrone (E1) metabolite; 4-OH metabolites are the most carcinogenic phase 1 metabolites.

4-Hydroxy-Estradiol (4-OH-E2)

Phase 1 estradiol (E2) metabolite; 4-OH metabolites are the most carcinogenic phase 1 metabolites.

16-Hydroxy-Estrone (16-OH-E1)

Phase 1 estrone (E1) metabolite; proliferative estrogen that may contribute to breast tenderness and heavy bleeding, etc.

2-Methoxy-Estrone (2-Me-E1)

Phase 2 metabolite that is much more stable than its precursor 2-OH-E1.

Total Estrogen

Represents the sum of all estrogen markers.

DHEA-S

Sulfated and circulating form of DHEA.

Androsterone

Alpha DHEA metabolite that is roughly seven times weaker than testosterone; can be used to estimate androgen activity in the tissues.

Etiocholanolone

Weak beta DHEA metabolite.

Testosterone

The major circulating androgen.

5a-DHT

Alpha metabolite of testosterone and the most potent androgen; roughly three to four times more androgenic than testosterone.

5a-Androstanediol

The best marker of 5a-DHT formation and activity in the tissues; can be used to estimate androgen activity in the tissues.

5b-Androstanediol

Weak beta androgen metabolite of testosterone that does not contribute to symptoms of androgen excess.

Epi-Testosterone

Not an androgenic hormone but produced in approximately the same amount as testosterone by the testes. Can be used to distinguish a truly low testosterone from a falsely low measurement due to the UGT genetic variant, see **"UGT Deletion" on page 31** for more information.

Creatinine

A compound that is produced from the metabolism of creatine from the kidneys and excreted in the urine. In order to provide the most accurate results, the DUTCH Test uses creatinine excretion to control for hydration status.

Free Cortisol Diurnal Pattern

Daily diurnal (circadian) rhythm of active cortisol that is normally highest in the morning and lowest at night.

Free Cortisone Diurnal Pattern

Daily diurnal (circadian) rhythm of inactive cortisone that is normally highest in the morning and lowest at night. The free cortisone diurnal pattern is used in combination with the free cortisol diurnal pattern to evaluate active free cortisol levels throughout the day, see **"The Cortisone Shadow" on page 122**, for more information.

Cortisol Awakening Response (CAR)

An HPA axis resiliency marker, an anticipatory marker of upcoming daily events, and a surrogate marker for HPA axis plasticity/reactivity.

Cortisol Clearance Rate (CCR)

Estimated rate that free cortisol and cortisone are cleared from circulation. Represents the ratio of (THF + THE)/(Total Free Cortisol + Total Free Cortisone).

Optional Extra Cortisol Sample (Insomnia Sample)

A measurement of cortisol upon waking at night.

a-Tetrahydrocortisol (a-THF)

Alpha cortisol metabolite.

b-Tetrahydrocortisol (b-THF)

Beta cortisol metabolite.

b-Tetrahydrocortisone (b-THE)

Beta cortisone metabolite.

Metabolized Cortisol (THE + THF)

Sum of all cortisol and cortisone metabolites.

Cortisol Clearance Rate (CCR)

Estimated rate that free cortisol and cortisone are cleared from circulation. Represents the ratio of (THF + THE)/(Total Free Cortisol + Total Free Cortisone).

Methylmalonate (MMA)

Metabolic byproduct of the citric acid cycle that elevates with vitamin B12 deficiency.

Xanthurenate

Metabolic byproduct of tryptophan metabolism that elevates with vitamin B6 deficiency.

Kynurenate

Metabolic byproduct of tryptophan metabolism that elevates with vitamin B6 deficiency and inflammation.

Pyroglutamate

An intermediate in glutathione recycling and production that elevates with glutathione deficiency.

b-Hydroxyisovalerate

Marker associated with biotin deficiency.

Indican

Metabolic byproduct of tryptophan metabolism in the gut that elevates with intestinal dysbiosis.

Homovanillate (HVA)

Primary dopamine metabolite.

Vanilmandelate (VMA)

Primary norepinephrine and epinephrine metabolite.

Quinolinate

Metabolic byproduct of tryptophan metabolism and neurotoxin that elevates with neuroinflammation and general inflammation.

Melatonin (6-OH-Melatonin Sulfate)

Melatonin metabolite.

8-Hydroxy-2-Deoxyguanosine (8-OHdG)

Marker associated with oxidative stress and DNA damage.

CAVEATS

TO TEST OR NOT TO TEST?

In some scenarios, DUTCH testing may have limited value. The following list discusses the most common scenarios that cause our providers to ask the question "to test or not to test?" If there is a scenario not listed below, please feel free to contact our lab for further guidance.

Precision Analytical does not recommend that a patient discontinue any medications or supplements prior to collection unless directed by their healthcare provider. Abruptly stopping some of the medications listed below could result in dangerous withdrawal syndromes.

MEDICATIONS

Most of the medications listed below have a physiologic effect on the body that can be evaluated using the DUTCH Test. However, a few medications may elevate select DUTCH biomarkers to the point where they are no longer clinically relevant; These are labeled below with an **"Affects Clinical Relevancy"** tag. In these cases the DUTCH Test cannot be used to evaluate the biomarkers that are directly affected.

The "time to baseline" refers to the amount of time it may take for a patient's DUTCH Test markers to return to baseline after stopping a medication or supplement. "Time to baseline" is a rough estimation and may be different for every patient.

5a-Reductase Inhibitors

Finasteride, Dutasteride, etc.

5a-reductase inhibitors inhibit the 5a-reductase enzyme and may lower alpha metabolites (androsterone, 5a-DHT, 5a-androstanediol, 5a-pregnanediol, 5a-THF) from baseline. Time to baseline: 4 weeks.

Amphetamines and Amphetamine-Like Medications and Drugs

Amphetamine, Dextroamphetamine, etc.

Amphetamines and amphetamine-like medications and drugs may increase free cortisol and free cortisone, HVA, and/or VMA from baseline. Time to baseline: These are eliminated from the body within 1 day, but it may take weeks to return to neurochemical baseline.

NOTE

Stimulant withdrawal may alter neurochemistry and result in severe side effects. Medical management of discontinuation is often required.

Antibiotics

Penicillins, Cephalosporins, etc

Infections, acute or chronic, that lead to the use of antibiotics may indirectly alter cortisol levels and cortisol metabolism from baseline. Time to baseline: 2 weeks after the infection has resolved.

Appetite Suppressants

Semaglutide, Naltrexone-Bupropion, Phentermine-Topiramate, etc.

Appetite suppressants, depending on the medication, may alter cortisol, HVA, and VMA from baseline. Time to baseline: depending on the medication, days to 2+ weeks.

Aromatase Inhibitors

Anastrazole, Letrozole, etc.

Aromatase inhibitors inhibit the aromatase enzyme and may directly lower all estrogens (and estrogen metabolites) and increase testosterone (and testosterone metabolites), and total DHEA from baseline. Time to baseline: 4-6 weeks.

Caffeine

Caffeine may indirectly increase free cortisol and cortisone, and possibly HVA and VMA from baseline. If the patient stops caffeine during collection and experiences a withdrawal effect, this may result in high free cortisol and free cortisone. Thus, if caffeine is a normal part of the patient's daily routine, consider not stopping it during collection (and if doing a DUTCH Plus or DUTCH CAR drink it after the +60-minute salivary sample). Note that coffee counts towards the total fluids consumed during the collection period. Time to baseline: Caffeine is eliminated from the body within 1 day, but it may take weeks to return to neurochemical baseline.

Clomiphene (Clomid)

In females, clomiphene may increase luteal progesterone, estrogens, and testosterone (and their metabolites) from baseline. In males it may increase testosterone and estrogens (and their metabolites) from baseline. Time to baseline: 3 cycles or 3 months, whichever comes first (cycling females); 5 weeks (males).

Synthetic Corticosteroids (Non-Hydrocortisone)

Time to baseline depends on dose, strength, frequency, and time since last dose.

- **Oral Corticosteroids** (e.g., Prednisone, Prednisolone, Methylprednisolone, etc.) Oral corticosteroids lower cortisol to nearly zero values and decrease metabolized cortisol and DHEA significantly from baseline. Time to baseline: 4-6+ weeks. Abruptly stopping oral corticosteroids could result in dangerous withdrawal symptoms.
- **Topical Corticosteroids** (e.g., Triamcinolone, Clobetasol, Mometasone, etc.) Topical corticosteroids have little to no effect on DUTCH markers, however, occasionally may lower cortisol and DHEA, but only if used on large areas of the body that results in high systemic absorption. Time to baseline: 2-6 weeks, depending on extent of use.
- **Inhaled Corticosteroids** (e.g., Fluticasone, Mometasone, Budesonide, Triamcinolone, etc.) Inhaled corticosteroids have little to no effect on DUTCH markers, however, occasionally may lower cortisol and DHEA. Time to baseline: 1-4 weeks depending on extent of use.

Diabetes Medications

Glucophage, Insulin, etc.

Diabetes medications may indirectly improve androgens, cortisol, cortisol/cortisone balance, Xanthurenate, and kynurenate levels from baseline. Time to baseline: varies depending on the medication, days to 2+ weeks.

Dopamine Reuptake Inhibitors (DRIs)

Wellbutrin/Bupropion, etc.)

DRIs may increase HVA and DHEA-S from baseline. It is unknown to what extent medications that affect neurotransmitter levels in the body will affect the neurotransmitter metabolites, HVA and VMA, on the DUTCH Test. Caution is advised regarding interpretation, as these medications may make the HVA and VMA results less clinically significant. Time to baseline: 6+ weeks.

Ephedrine (including Sudafed)

Ephedrine may indirectly increase cortisol and VMA from baseline. Time to baseline: 30-80 hours.

Fertility Medications

- See **Aromatase Inhibitors**, Clomiphene, Follicle Stimulants, hCG, and GnRH Analogs. Time to baseline: 3 cycles or 3 months, whichever comes first (cycling females).
- **Follicle Stimulants** (e.g., Follitropin Beta (Follistim), Follitropin Alfa (Gonal-F), etc.) Follicle stimulants increase estrogen and estrogen metabolites from baseline. Time to baseline: 3 cycles or 3 months, whichever comes first.
- **GnRH Analogs** (e.g., Triptorelin, Goserelin, Leuprorelin, etc.) GnRH analogs may indirectly lower estrogens and estrogen metabolites during treatment. Time to baseline: 3 cycles or 3 months, whichever comes first.

Hormonal Birth Control

Time to baseline: 3 cycles or 3 months, whichever comes first.

- **Combination Contraceptives & Progestin-Only Injections** (e.g., Depo-Provera)
- Combination contraceptives that contain a progestin + ethinyl estradiol:
  - **Combination Oral Contraceptives (COCs)** (e.g., Ortho-Tri Cyclen, Yaz, Yasmin, etc.)
  - **Vaginal Rings** (e.g., NuvaRing)
  - **Birth Control Patches** (e.g., Xulane)

These all suppress the hypothalamic-pituitary-ovarian (HPO) axis, resulting in consistently low endogenous progesterone and estrogen levels. The DUTCH Test does not measure the synthetic hormones in these forms of birth control. You will be able to see estrogen metabolism patterns for endogenous estrogens but not for synthetic estrogen (i.e., ethinyl estradiol). Apart from progesterone and estrogen, the DUTCH Test also includes androgens, cortisol, and organic acids, so some females may still benefit from running a DUTCH Plus® or DUTCH Complete™. The DUTCH Cycle Mapping™ does not offer clinically relevant information in females on these forms of birth control.

Precision Analytical does not recommend that a patient discontinue any medications or supplements prior to collection unless directed by their healthcare provider. Abruptly stopping some of the medications listed below could result in dangerous withdrawal syndromes.

- **Progestin-Only Pills (POPs – "Minipill") & Progestin Implants** (e.g., Nexplanon) These often suppress ovulation and progesterone; however some females still ovulate. The DUTCH Test does not measure the synthetic progestin in these POPs and implants. Apart from progesterone and estrogen, the DUTCH Test also includes androgens, cortisol, and organic acids, so some females may still benefit from running a DUTCH Plus® or DUTCH Complete™. The DUTCH Cycle Mapping™ may not offer clinically relevant information in females on these forms of birth control if the birth control is suppressing estrogen and progesterone production from the ovaries. POPs that contain drospirenone are more likely to suppress ovulation than ones containing norethindrone.

- **Hormonal IUDs** (e.g., Mirena, Kyleena, Skyla, Liletta, etc.) These are progestin containing intrauterine devices that sometimes suppress ovulation and progesterone; however some females still ovulate and cycle regularly with a hormonal IUD. While controversial, we have also seen anecdotally that patients on hormonal IUDs may experience estrogen dominance, and this state can be observed on a DUTCH Test. Some females may benefit from the DUTCH Complete™ or DUTCH Plus®. If the person is still cycling with the hormonal IUD, then the DUTCH Cycle Mapping™ may also have clinical utility, however, keep in mind that the progestin in the IUD can influence progesterone and/or estrogen levels.

Bioidentical Hormone Replacement Therapy (BHRT)

See page **page 24** for more information about monitoring BHRT with DUTCH Testing. Time to baseline: 7-14 days.

- **Oral Bioidentical Hormones Affects Clinical Relevancy**
  Due to the first pass metabolism effect in the gut and liver, oral hormones result in elevated urinary hormone levels that do not reflect circulating hormone levels if taken within 72 hours of sample collection. Thus, adjusting HRT dosing based on DUTCH Test results is not advisable as the results are a combination of first-pass metabolites plus the true value in circulation. However, hormone metabolism patterns (as represented by the population sliders and pie chart) are still accurate. For example, a provider may still use the DUTCH test to assess a patient's a- to b-pregnanediol balance, 2-OH/4-OH-E1 balance, 2-OH/16-OH-E1 balance, methylation activity, and 5a-preference.

NOTE

A note about oral progesterone: When a female patient reports taking oral progesterone within 72 hours of sample collection, we adjust the progesterone reference ranges on a female report to reflect the a-pregnanediol and b-pregnanediol levels that are typically seen when a standard dose of 100-200 mg is taken during collection (and not skipped). Progesterone that is within the oral progesterone reference range does not guarantee that the dose and route of administration (ROA) of progesterone is appropriate for endometrial protection when estrogen replacement therapy (ERT) is being used concomitantly. When oral progesterone is supplemented, providers can focus on the a- to b-pregnanediol balance which can offer insights on the role of oral progesterone on mood and sleep.

- **Sublingual Bioidentical Hormones Affects Clinical Relevancy**
  The portion of the sublingual hormones that is swallowed will elevate urine hormone metabolites due to the first-pass effect in the gut and liver if the sublingual hormones are taken within 72 hours of sample collection. Subsequently, urine results will not correlate with serum results when sublingual hormones are taken within 72 hours of sample collection. However, hormone metabolism patterns (as represented by the population sliders and pie chart) are still accurate. For example, a provider may still use the DUTCH test to assess a patient's a- to b-pregnanediol balance, 2-OH/4-OH-E1 balance, 2-OH/16-OH-E1 balance, methylation activity, and 5a-preference.

- **Transdermal (Topically to the Skin) Bioidentical Hormones**
  The DUTCH Test results accurately reflect circulating levels of hormones when this route of administration is used.

- **Vaginal Bioidentical Hormones**
  The DUTCH Test is unique in that the free hormones are separated from the conjugated hormones prior to analysis allowing for accurate testing even with the use of intravaginal hormones that may contaminate the urine. The DUTCH Test results accurately reflect circulating levels of hormones but may not represent uterine concentrations of these hormones. Note that vaginal bioidentical hormones only affect clinical relevancy of the DUTCH Test results if there is *extreme* contamination of the urine sample paper, which is rare.

- **Bioidentical Hormone Pellets**
  The DUTCH Test can be used to monitor estradiol levels when estradiol pellets are used, as urine correlates well with serum. Testosterone pellets increase the DUTCH Test urinary testosterone levels, as expected, but the increase may exceed what is seen in serum testing.

- **Injected Bioidentical Testosterone**
  Intramuscular (IM) testosterone injections increase the DUTCH Test urinary testosterone levels, as expected, but the increase may exceed what is seen in serum testing.

Human Chorionic Gonadotropin (hCG)

Ephedrine may indirectly increase cortisol and VMA from baseline. Time to baseline: 30-80 hours.

Hydrocortisone

Despite its name, hydrocortisone is bioidentical cortisol.

- **Hydrocortisone Cream May Affect Clinical Relevancy**

  Hydrocortisone cream may affect the clinical relevancy of the free cortisol results if it contaminates the samples. When a person touches the samples with hydrocortisone cream on their hands (even a little), it can contaminate them. Cortisol must enter the body in order to be deactivated to cortisone, thus when the samples are contaminated by hydrocortisone cream, the free cortisol will be affected but the free cortisone won't. In these cases, the free cortisone pattern is a better representation of the diurnal pattern. Time to baseline: avoid exposure to and use of on the day(s) of collection to avoid contamination.

Precision Analytical does not recommend that a patient discontinue any medications or supplements prior to collection unless directed by their healthcare provider. Abruptly stopping some of the medications listed below could result in dangerous withdrawal syndromes.

- **Hydrocortisone Injections**
  Hydrocortisone injections directly increase free cortisol and free cortisone during the 1-2 days after injection, however, after a few days they may result in adrenal suppression and very low cortisol (and lower total DHEA) from baseline. Time to baseline: 6-8 weeks.

- **Oral Hydrocortisone May Affect Clinical Relevancy**
  Oral hydrocortisone directly increases free cortisol and free cortisone for 4-6 hours after supplementation and metabolized cortisol (THE + THF) for 10-12 hours after supplementation. Oral hydrocortisone only affects the clinical relevancy of the free cortisol if it contaminates the samples. Cortisol must enter the body in order to be deactivated to cortisone, thus when the samples are contaminated by hydrocortisone, the free cortisol will be affected but the free cortisone won't. In these cases, the free cortisone pattern is a better representation of the diurnal pattern. Time to baseline: 10-12 hours. Abruptly stopping oral corticosteroids could result in dangerous withdrawal symptoms, depending on the dose and frequency used.

Levodopa Affects Clinical Relevancy

Levodopa results in elevated HVA (and possibly VMA) levels that are not indicative of high dopamine (or high norepinephrine and epinephrine) in the body. Time to baseline: 48 hours.

Methyltestosterone

Methyltestosterone is a synthetic anabolic steroid that does not show up on the DUTCH Test as testosterone or its metabolites. In males, methyltestosterone is used as a replacement therapy in testosterone deficiency conditions, such as hypogonadism. It suppresses endogenous production of testosterone. Thus, on the DUTCH Test it may lower testosterone and its metabolites, and epi-testosterone from baseline. In females, methyltestosterone is used in certain types of breast cancer treatment. Time to baseline: females usually test while on methyltestosterone. In males, it is eliminated from the body within 15 hours, however time to true baseline may take weeks.

Opioid Pain Medications

Oxycodone, Hydrocodone, Morphine, etc.

Opioid medications may indirectly decrease sex hormones, DHEA and cortisol from baseline. Time to baseline: 6-12 weeks (males and non-cycling females); 3 cycles or 3 months, whichever comes first (cycling females). Note: Opioid withdrawal may alter neurochemistry and result in severe side effects. Medical management of discontinuation is required.

Selective Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs)

Venlafaxine, Desvenlafaxine, Duloxetine, etc.

SNRIs may improve the cortisol awakening response (CAR) from baseline in some patients. It is unknown to what extent medications that affect neurotransmitter levels in the body will affect the neurotransmitter metabolites, HVA and VMA, on the DUTCH Test. Caution is advised regarding interpretation, as these medications may make the HVA and VMA results less clinically significant. Time to baseline: 6+ weeks.

Selective Serotonin Reuptake Inhibitors (SSRIs)

Fluoxetine, Citalopram, Sertraline, etc.

SSRIs may improve the cortisol awakening response (CAR) from baseline in some patients. Caveat about fluoxetine: may decrease CYP3A4 from baseline. It is unknown to what extent medications that affect neurotransmitter levels in the body will affect the neurotransmitter metabolites, HVA and VMA, on the DUTCH Test. Caution is advised regarding interpretation, as these medications may make the HVA and VMA results less clinically significant. Time to baseline: 6+ weeks.

Spironolactone

Spironolactone may lower testosterone and estrogen (and their metabolites) from baseline. It may increase DHEA-S secondary to increasing SHBG and prolactin. Moreover, it may increase 11b-HSD2 activity and increase free cortisone while lowering free cortisol from baseline. Time to baseline: Spironolactone's effects on SHBG and prolactin may be persistent for weeks (perhaps 2-6 weeks). If true baseline is desired, the return of original symptoms (e.g., acne, hirsutism, etc.) may be the clearest sign.

Tamoxifen

Tamoxifen is a **Selective Estrogen Receptor Modulator (SERM)** that may increase estrogens and their metabolites from baseline (mostly seen in premenopausal cycling females). Time to baseline: 10 weeks (non-cycling females); 3 cycles or 3 months, whichever comes first (cycling females).

Thyroid Medication

T3, T4

Patients typically take their thyroid medication as usual during collection.

Tricyclic Antidepressants (TCAs)

Amitriptyline, Nortriptyline, Imipramine, etc.

TCAs may alter HVA and VMA from baseline. It is unknown to what extent medications that affect neurotransmitter levels in the body will affect the neurotransmitter metabolites, HVA and VMA, on the DUTCH Test. Caution is advised regarding interpretation, as these medications may make the HVA and VMA results less clinically significant. Time to baseline: 6+ weeks.

Precision Analytical does not recommend that a patient discontinue any medications or supplements prior to collection unless directed by their healthcare provider. Abruptly stopping some of the medications listed below could result in dangerous withdrawal syndromes.

SUPPLEMENTS

Precision Analytical does not recommend that a patient discontinue any medications or supplements prior to collection unless directed by their healthcare provider.

All the supplements listed below (except DIM and I3C) can elevate select DUTCH biomarkers to the point where they are no longer clinically relevant; These are labeled below with an **"Affects Clinical Relevancy"** tag. DIM and I3C have a physiological effect on the body that can be evaluated using the DUTCH Test.

The "time to baseline" refers to the amount of time it may take for a patient's DUTCH Test markers to return to baseline after stopping a medication or supplement. "Time to baseline" is a rough estimation and may be different for every patient.

Diindolylmethane/Indole-3-Carbinol (DIM/I3C)

DIM and I3C tend to lower circulating parent estrogen levels (E1 and E2) and encourage more estrogen down the preferred 2-OH pathway. Time to baseline: 24 hours+ (non-cycling females and males); 3 cycles or 3 months, whichever comes first (cycling females).

D,L-Phenylalanine (DLPA) Affects Clinical Relevancy

DLPA results in elevated HVA (and possibly VMA) levels that are not indicative of high dopamine (or high norepinephrine and epinephrine) in the body. Time to baseline: 48 hours.

Hydroxymethylbutyrate (HMB) Affects Clinical Relevancy

HMB results in elevated b-hydroxyisovalerate (the DUTCH Test biotin marker) that is not indicative of a biotin deficiency. Time to baseline: 48 hours.

L-Dopa Affects Clinical Relevancy

L-Dopa results in elevated HVA (and possibly VMA) levels that are not indicative of high dopamine (or high norepinephrine and epinephrine) in the body. Time to baseline: 48 hours.

Melatonin Affects Clinical Relevancy

Oral melatonin directly affects the DUTCH melatonin metabolite, 6-OH-melatonin sulfate. Due to the first pass metabolism effect in the gut and liver, oral melatonin results in elevated urinary melatonin levels that do not reflect circulating melatonin levels. The DUTCH Test cannot be used to determine if the oral melatonin dose is appropriate. The DUTCH melatonin biomarker is only clinically useful as a baseline marker when melatonin is not taken during the night(s) before collection. Time to baseline: depending on dose 1-3 days.

Mucuna Affects Clinical Relevancy

Mucuna is an herb that contains L-Dopa and results in elevated HVA (and possibly VMA) levels that are not indicative of high dopamine (or high norepinephrine and epinephrine) in the body. Time to baseline: 48 hours.

Phenylalanine Affects Clinical Relevancy

Phenylalanine results in elevated HVA (and possibly VMA) levels that are not indicative of high dopamine (or high norepinephrine and epinephrine) in the body. Time to baseline: 48 hours.

Quercetin Affects Clinical Relevancy

Quercetin results in elevated HVA which is not indicative of high dopamine in the body. Time to baseline: 48 hours.

Tryptophan Affects Clinical Relevancy

Oral tryptophan results in elevated xanthurenate, kynurenate, and quinolinate that are not indicative of a vitamin B6 deficiency or neuroinflammation. Time to baseline: 48 hours.

Tyrosine Affects Clinical Relevancy

Oral tyrosine results in elevated HVA and VMA levels that are not indicative of high dopamine, norepinephrine, and epinephrine in the body. Time to baseline: 48 hours.

DAMP, WET, AND MOLDY URINE SAMPLES

It is important to *completely* dry all DUTCH urine collection strips. When the DUTCH urine collection strips are not properly dried and remain damp, wet, or moldy during transit, analyte stability can decrease. Potential analyte degradation is dependent on the amount of moisture (damp vs. wet), length of time the sample(s) were damp or wet, the degree to which mold has developed, and on the individual urine matrix. Not all analytes degrade in these situations; For example, when urine samples begin to mold, select OATS analytes can degrade, while others can be enhanced. If leaving the samples out to air dry is not possible (not enough time, high humidity, etc.), a hair dryer can be used effectively without using excessive heat settings.

PHYSICAL STATE

Amenorrhea

Females who do not have a period because their ovaries are not cycling likely have low estrogen and progesterone throughout the month. A DUTCH Complete or DUTCH Plus may be helpful in this scenario, and the person can test on any day. The DUTCH Cycle Mapping does not offer clinically relevant information in females with amenorrhea because their hormones do not “cycle.”

Partial Hysterectomy

Females who do not have a period due to a prior hysterectomy (but still have at least one ovary) can still collect for a DUTCH Plus, DUTCH Complete, and/or DUTCH Cycle Mapping. For the DUTCH Complete and DUTCH Plus panels, we suggest collecting 5-7 days after ovulation. Ovulation can be determined by following basal body temperature (BBT) and by using an ovulation predictor kit (OPK).

NOTE

> *If the patient is postmenopausal, a DUTCH Cycle Mapping test will not offer clinically relevant information (due to the patient no longer cycling).*

Complete Hysterectomy

Females who have had a complete hysterectomy (no ovaries remaining) likely have postmenopausal levels of estrogen and progesterone. A DUTCH Complete or DUTCH Plus may offer more clinically relevant information in this scenario. DUTCH Cycle Mapping does not offer clinically relevant information in people who have had a complete hysterectomy.

Pregnancy

The DUTCH Test does not have reference ranges for pregnant women; therefore, clinical utility may be limited if testing during pregnancy.

Illness

Acute illness (common cold, urinary tract infection, etc.) can affect cortisol levels and cortisol metabolism. Testing may be better utilized upon full recovery.

Kidney Disease

Kidney disease can affect the accuracy of DUTCH Test results. If glomerular filtration rate (GFR) and creatinine on serum testing are normal, then DUTCH testing is appropriate. Note that this only pertains to urine samples; a person with kidney disease can still collect accurate data from salivary samples with a DUTCH CAR or DUTCH Plus panel.

UGT DELETION

Some people are born with a genetic deletion that causes their testosterone, 5a-DHT, and 5b-androstanediol to be falsely low in the urine. This genetic difference is known as the UGT2B17 deletion.

The DUTCH Test measures urinary testosterone glucuronide and testosterone sulfate. These testosterone conjugates are formed mostly from bioavailable testosterone that undergoes phase 2 metabolism to make them ready for urinary excretion.

Testosterone, in the liver, is primarily glucuronidated by the UGT2B17 enzyme, which also produces 5a-DHT and 5b-androstanediol glucuronides. Genetic variants in this enzyme reduce these urinary hormone levels without affecting serum levels.

The genetic UGT2B17 variants vary in the population, with a higher incidence seen in individuals of South Asian descent. The literature continues to evolve, noting a higher incidence in the UGT2B17 variants in non-Asian populations. Heterozygous individuals show milder reductions in urinary testosterone levels than homozygous individuals.

For this reason, low and very low urinary testosterone levels should be confirmed with serum testing before treatment. Serum testing should include total testosterone, free testosterone (either calculated or measured by equilibrium dialysis), and sex hormone binding globulin (SHBG).

Epi-Testosterone May Help Identify the UGT Deletion Pattern

Epi-Testosterone is not androgenic but is included on the DUTCH Test because it may be helpful when assessing the validity of the urinary testosterone result. In males, epi-testosterone is made in the testes in about the same concentration as testosterone. Similarly, in females, epi-testosterone is made in the ovaries in about the same concentration as testosterone. Thus, if epi-testosterone is much higher than testosterone, 5a-DHT, and 5b-androstanediol, the UGT deletion may be present, and testosterone should be measured in serum.

Epi-Testosterone can be suppressed if a person is using testosterone replacement therapy (TRT) as TRT can suppress testicular or ovarian production of testosterone (and thus epi-testosterone). In these cases, epi-testosterone can be used to estimate endogenous testosterone production from the testes or ovaries when testosterone is being supplemented.

NOTE

> *Epi-Testosterone is a less reliable marker of testosterone production in women who are taking oral contraceptives and in women after menopause. Women taking oral contraceptives have been found to have 40% less epi-testosterone in their urine and epi-testosterone drops more sharply than testosterone during the menopause transition.*

ADDITIONAL SCENARIOS

Children

DUTCH Test is not intended for prepubescent individuals, the reference ranges are established for adult populations only. Androgen and estrogen levels are often much lower in children. In order to provide the most accurate results, the DUTCH Test uses creatinine excretion to control for hydration status. DUTCH recommends against testing urine samples from children under the age of 12.

NOTE

> *Children between the ages of 9-12 can still collect accurate data from salivary samples with a DUTCH CAR or DUTCH Plus panel.*

Some clinical situations (such as suspected precocious puberty), providers may wish to order a DUTCH urine panel for a child between the ages of 9-12. DUTCH will process these samples but does not have adjusted reference ranges for children in this age range, so interpretation must be completed with that consideration in mind.

Steroid Medications

Steroid medications (e.g., prednisone, budesonide, steroid inhalers, cortisone injections) can suppress the HPA axis. The degree of suppression depends upon the medication strength, dose, frequency of use, duration of use, and time since last use. Adrenal function usually recovers in 4-12 weeks; however, any medication adjustments should be discussed with a physician. The DUTCH Complete, DUTCH Plus and/or DUTCH Cycle Mapping may be helpful to see the extent of adrenal suppression, and how adrenal suppression is affecting androgen levels (e.g., DHEA, testosterone, etc.).

WHEN TO COLLECT

CYCLING FEMALES

Cycling females may consider collecting in their midluteal phase, between days 19 and 22 of a 28-day cycle, see **Figure 1.12**. If the cycle is different than 28 days in length, simply subtract 6-9 from the cycle length. For example: collect on days 17-20 for a 26-day cycle. If there are irregular cycles, consider collecting 5-7 days after ovulation. Ovulation can be determined by following BBT and with an OPK, see **Figure 1.13** as well as **"Basal Body Temperature (BBT)"** and **"Ovulation Predictor Kits (OPKs)"** below, to learn how to use BBT and OPKs. It may be helpful to collect in the luteal phase to understand if ovulation has occurred, if the corpus luteum is making adequate progesterone, and if estrogen is well balanced with progesterone.

DUTCH Collection Tips

If the person is not sure they collected at the right time, they can consider air-drying the urine strips for 24 hours and then placing them in their freezer until they get their period. If they've successfully collected their samples at the right time in their cycle, then they should get their period no less than 4 days, and no more than 10 days after collection. For example, if they collected on January 1st (and collected at the right time in their cycle), then they should start their period sometime between January 5th and 11th.

Short Cycles

If the person's cycles are 20 days in length or shorter, they may consider collecting 5-7 days prior to the onset of their menses.

Long Cycles

If someone has very long cycles, they may consider using an ovulation predictor kit (OPK), and start testing for ovulation at any time. They can consider testing daily to see if they get a positive result. If their period starts before they get a positive result, then they can consider resuming OPK testing as soon as their period has stopped, or when the bleeding has slowed down to just spotting. Some females with very long cycles may benefit from testing on any day instead of timing collection with ovulation.

Calculating Cycle Length

A female's cycle length is the number of days from the day their period starts to the day before their next period starts. For example, if their period starts on January 1st and their next period starts on January 29th, then they had a 28-day cycle.

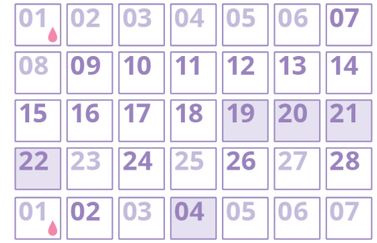
Cycling Females without a Period

If the patient doesn't get a period (Hysterectomy, Ablation, Etc.) but their ovaries are still cycling, then they can use an OPK to determine when they ovulate. They may consider starting to use the OPK on any day and test daily to see if they get a positive result. Once they get a positive result, they may collect 5-7 days later.

Other Ways to Determine Ovulation

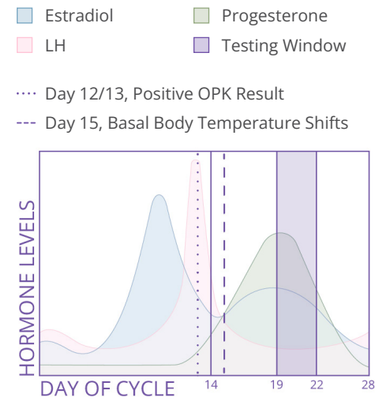
Some females will track their Basal Body Temperature (BBT) to help determine when they ovulate. Other females will monitor for signs of ovulation, such as cervical mucus consistency and the position and firmness of their cervix.

Figure 1.12: Collection Scheduling for Cycling Females



The schedule on which a patient collects depends on the average length of their cycle. If the patient has a standard 28-day cycle, they may consider collecting between days 19 and 22 for a DUTCH Complete or DUTCH Plus. For DUTCH Cycle Mapping, the patient will begin collecting on the seventh day of the cycle, collecting every dark purple day, preferably in the morning. On the fourth day of the new cycle, they will collect the final four samples.

Figure 1.13: Identifying the Testing Window with OPKs or BBT



In a 28-day cycle, it is recommended to collect between days 19 and 22. Ovulation is marked at day 14 after a spike in luteinizing hormone (LH). An Ovulation Predictor Kit (OPK) will have a positive result approximately 1.5 days before ovulation, on day 12 or 13, and Basal Body Temperature (BBT) will shift one day after ovulation, on day 15.

POSTMENOPAUSAL FEMALES

Postmenopausal females can collect on any day. If the DUTCH Test panel includes cortisol, consider collecting on a normal day to best measure typical adrenal function.

MALES

Males can collect on any day. If the DUTCH Test panel includes cortisol, consider collecting on a normal day to best measure typical adrenal function.

OVULATION PREDICTOR KITS (OPKS)

It may be clinically useful for cycling females to collect their DUTCH urine samples during their midluteal phase, about 5-7 days after ovulation. OPKs can help to identify what day ovulation takes place. BBT can also help, see **“Basal Body Temperature (BBT)” on page 35** for more information.

Where to Buy

OPKs can be purchased online or at many grocery stores and pharmacies.

Confirming Ovulation

OPKs detect the presence of luteinizing hormone (LH) in the urine. LH triggers the ovary to release an egg. A strong positive result means that LH is surging, and ovulation will likely happen within the next 8-20 hours.

When to Test

The person should always defer to the OPK’s instructions. However, as a general rule, it is best practice to start the OPK strips 18 days prior to menstruation, taking into account the shortest cycle length. For example, if cycles range from 23-35 days, it is best practice to start OPK strips on cycle day five. To calculate this, we used the shortest cycle length (23 days) and subtracted 18. Always defer to the OPK’s instructions, however research supports collection in the late morning, around 10am.

When to Collect DUTCH Samples

A positive result means that LH is peaking, and the person will likely ovulate within 8-20 hours. Consider collecting the DUTCH urine samples 5-7 days after ovulation, or roughly 5-7 days after a positive OPK result.

Special Considerations

Women with PCOS and perimenopausal females should use caution with OPKs, as they may get a positive result but not ovulate. If someone still decides to utilize OPKs to time their collection, please see **“DUTCH Collection Tips” on page 33** for more information.

Troubleshooting

If the person has tested for 60 days and hasn’t gotten a positive result, they may consider collecting their samples on any day.

BASAL BODY TEMPERATURE (BBT)

It might be clinically useful for cycling females to collect their DUTCH urine samples during their midluteal phase, which is about 5-7 days after ovulation. BBT can help one understand what day ovulation takes place. OPKs can also help, see **“Ovulation Predictor Kits (OPKs)” on page 34** for more information.

Confirming Ovulation

Preovulatory BBTs range from about 97.0 to 97.7 degrees Fahrenheit. After ovulation, BBT rises to about 97.8 degrees or higher. This is because after a female ovulates, the follicle that released the egg turns into the corpus luteum and begins making progesterone. Progesterone is thermogenic meaning it increases body temperature.

How to Measure

To measure BBT, take your oral temperature with a BBT thermometer each morning upon waking, ideally at the same time each day. A BBT thermometer is a more sensitive thermometer that reads to 2 decimal places and within the normal temperature ranges. Many tech wearables also will measure body temperature, allowing this measurement to occur without any additional effort, such an Oura Ring.

When to Collect DUTCH Samples

A person can consider charting their temperature on paper, or in an app such as Kindara, to look for patterns. It is helpful to look for patterns. Before ovulation, BBT may be lower and then jump up after ovulation and stay higher until the next cycle starts. If a temperature rise occurs for three days straight (especially if it rises by 0.5 degrees), then it is likely that ovulation occurred right before the rise in temperature. Collection of DUTCH samples 5-7 days after ovulation (or about 2-4 days later after the third day of elevated BBT readings) offers best clinical insight.

MONITORING (B)HRT WITH DUTCH

Figure 1.14: Monitoring (B)HRT

More information on HRT monitoring can be found on the DUTCH Education page at dutchtest.com. Consider reviewing the HRT Testing Matrix by scanning the above QR code (or visiting https://dutchtest.com/resource/testing-matrix/) for more information and to compare different lab methods.

OVERVIEW

A common use of the DUTCH Test is to evaluate and monitor patients on HRT. HRT most commonly consists of estrogen, progesterone, and testosterone, and can come in a variety of forms—some that are FDA-approved prescriptions and others that are custom compounded. To understand how the DUTCH Test can be most useful for HRT, review the following considerations for testing and interpretation.

NOTE

The DUTCH Test is not the best lab tool for all forms of hormone replacement but may offer valuable information regarding hormone metabolism patterns, see **Figure 1.14** *for more information.*

ESTROGEN THERAPY [B]

Using DUTCH for Estrogen Therapy

The best uses of the DUTCH Test with estrogen therapy include:

1. Evaluating estrogen detoxification pathways. For example, is estrogen being pushed into the carcinogenic 4-OH estrogen catechols that increase risk for breast cancer? Or is estrogen being pushed into the proliferative 16-OH-E1 metabolites that can contribute to heavy bleeding, breast tenderness, and fibroid growth?
2. Monitoring transdermal (TD) estrogen therapies (patches, creams, gels) and pellet therapy, as urine correlates well with serum and aligns with clinical outcomes when these routes of administration are utilized.

Transdermal (TD) Estrogen

The DUTCH Test can be used to monitor estradiol (E2) levels when estrogen is used transdermally, as urinary E2 correlates with serum E2 and aligns with clinical outcomes. Urine additionally provides information about estrogen metabolism patterns and may be a better representation of circulating E2 levels than serum, as it looks at an average over the course of collection and not one point in time.

NOTE

Transdermal estradiol (delivered via gel or cream) dosed between 0.25 – 1.0mg increases urinary E2 concentrations to levels observed with the use of transdermal estradiol patches dosed between 0.025-0.05mg, and urine levels have been shown to parallel serum levels.

[b] Newman MS, Curran DA, Mayfield BP, Saltiel D, Stanczyk FZ. Assessment of estrogen exposure from transdermal estradiol gel therapy with a dried urine assay. Steroids. 2022 Aug; 184:109038. doi: 10.1016/j.steroids.2022.109038. Epub 2022 Apr 26. PMID: 35483542.

DUTCH data for patients on this type of therapy were recently published in the peer-reviewed journal, Steroids, and presented at the 2021 North American Menopause Society (NAMS) meeting, see the data in **Figure 1.15** or find the full-text in **Figure 1.16**.

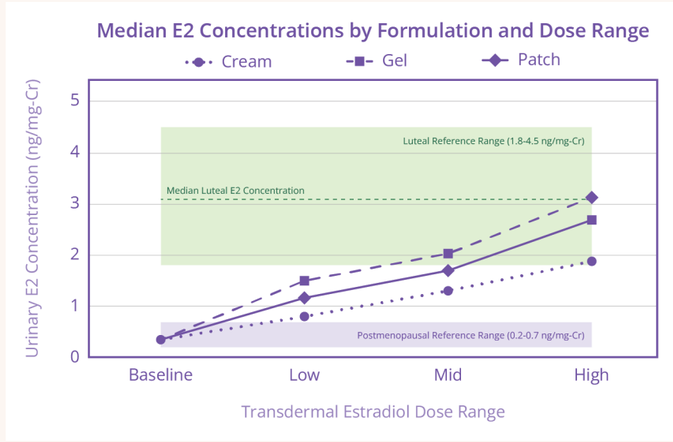


Figure 1.15: ***Median Estradiol Concentrations by Formulation and Dose Range***
Transdermal E2 product doses that increase E2 concentrations above the postmenopausal range are the doses that have been proven to have clinical impact.

Our data shows that transdermal E2 product doses that increase E2 concentrations above the postmenopausal range are the doses that have been proven to have clinical impact. It is not generally necessary to increase levels into the premenopausal (luteal) range to achieve some level of significant clinical change. Therefore, general recommendations for patients on this type of therapy is an E2 value between the postmenopausal and premenopausal range, depending on the clinical goals. A patient whose estrogen levels remain in or below the postmenopausal range for E2 may not have the same level of clinical improvement as a patient with a higher E2 concentration; however, each patient case, along with any risks associated with insufficient or excessive estrogen, should be considered individually.

Response to this type of therapy may vary from person to person as interindividual absorption variability has been shown to be high. This makes testing helpful in assessing an individual's estradiol exposure. Assessing estradiol levels, as well as monitoring downstream metabolites to assess phase I metabolism and methylation is vitally important for optimizing therapy. Precision Analytical does not endorse any particular dose or type of therapy for ERT.

Oral Estrogen

If estrogen is supplemented orally within 72 hours of sample collection, urine estrogen metabolites are expected to be elevated due to the first-pass effect in the gut and liver. Subsequently, urine results will not correlate with serum results when this route of administration is used.

Figure 1.16: DUTCH Research

DUTCH data for patients on transdermal estrogen was recently published in the peer-reviewed journal, Steroids, and presented at the 2021 North American Menopause Society (NAMS) meeting. Scan the QR code to find the full text of the manuscripts, or visit: (https://dutchtest.com/research/).

NOTE

If a patient is using oral estriol (E3) a portion of the E3 is metabolized into 16-OH-E1 in the gut and liver during first-pass and may result in an elevated 16-OH-E1 percentage relative to the 2-OH-E1 and 4-OH-E1 percentages. In this scenario, use care when interpreting results.

Sublingual Estrogen

If estrogen is supplemented sublingually within 72 hours of sample collection, the portion of the estrogen hormone that is swallowed will elevate urine estrogen metabolites due to the first-pass effect in the gut and liver. Subsequently, like oral estrogen, urine results will not correlate with serum results when sublingual estrogen is taken.

Vaginal Estrogen

There is often the concern that estrogen used vaginally may contaminate the urine and falsely elevate results. DUTCH testing is unique in that free hormones are separated from conjugated hormones prior to analysis allowing for accurate testing even with the use of intravaginal hormones.

The DUTCH results accurately reflect circulating levels of estrogen when the estrogen replacement therapy (ERT) is applied labially or inserted in the lower third of the vaginal vault.

NOTE

ERT inserted in the upper third of the vaginal vault may excessively elevate endometrial estrogen levels.

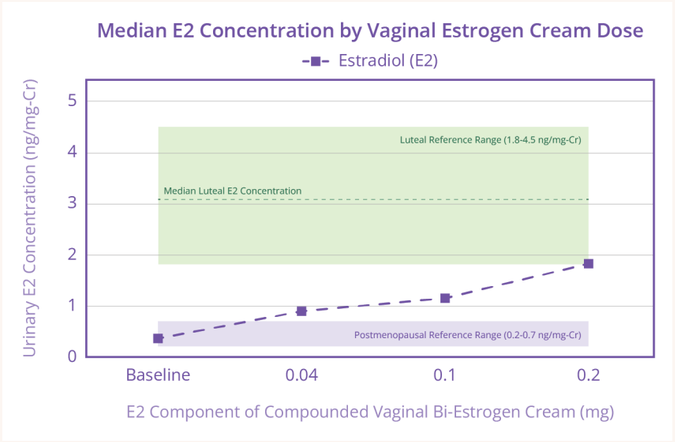


Figure 1.17: ***Median E2 Concentration by Vaginal Estrogen Cream Dose***
Increasing doses of vaginal bi-estrogen cream resulted in a dose-dependent increase in median urinary concentrations of E2.

In a recent North American Menopause Society (NAMS) publication [c], DUTCH showed increasing doses of vaginal estrogen cream resulted in a dose-dependent increase in median urinary concentrations of estrone (E1), estradiol (E2), and estriol (E3), see **Figure 1.17**.

NOTE

If the clinical goal is to provide therapy for a local, vaginal effect (and to avoid meaningful elevations of systemic estrogen), the goal should be for estradiol levels on DUTCH testing to stay within the postmenopausal range.

Estrogen Pellets

The DUTCH Test can be used to monitor estradiol (E2) levels when estrogen pellets are used, as urine correlates well with serum. If on a 3-month dosing schedule, consider testing halfway through dosing interval (6-8 weeks after insertion) and baseline trough levels (3-4 months after pellet insertion).

Rectal Estrogen

The DUTCH Test urinary estrogen levels may be higher than serum estrogen levels, as some of the estrogen may be transported to the liver via the portal vein and become metabolized.

PROGESTERONE THERAPY

Using DUTCH for Progesterone Therapy

Currently there is no lab testing that can be used to guarantee endometrial protection for patients on progesterone therapy. Studies that have assessed the progesterone dosing and routes of administration (ROA) required to protect the endometrium when concomitant ERT is being supplemented use endometrial biopsies to identify and categorize endometrial hyperplasia. As it is not always practical to perform endometrial biopsies on patients to monitor progesterone therapy, dosing and ROA are based on these studies, which have found that 100-200 mg of oral or 45-90 mg of vaginal progesterone, nightly, protects the endometrium.

Measuring Progesterone

Measuring oral progesterone is difficult because serum progesterone levels rapidly rise and fall within a few hours after dosing. Vaginal progesterone inserted into the upper third of the vaginal vault concentrates in the uterus, and serum levels do not reflect this concentration. Transdermal progesterone creams have not demonstrated the ability to reliably increase serum levels nor provide endometrial protection. Transdermal progesterone gels tend to absorb better than creams, but also haven't demonstrated the ability to inhibit endometrial proliferation.

Urine progesterone metabolites do not strongly correlate with serum progesterone with many common ROAs (oral, sublingual, topical, vaginal) thus it is not generally

[c] Mark Newman, MS; Doreen Saltiel, MD, JD; Desmond A Curran. "Approximating Systemic Estrogen Exposure from Vaginal Estrogen Cream Therapy." Poster presented at NAMS, 2022. Atlanta, GA.

recommended to use the DUTCH Test results to monitor circulating progesterone levels with progesterone therapy. Even a "within range" progesterone result on the DUTCH Test does not ensure endometrial protection.

One unique feature of the DUTCH Test is that it measures progesterone metabolites, which can be useful for assessing other benefits of progesterone therapy. Patients who push progesterone more heavily down the 5a pathway (towards a-pregnanediol) have been shown to make more of the other 5a metabolites like allopregnanolone, which modulate GABA receptors in the brain and can support mood and sleep. See **"Progesterone Metabolism" on page 84** for more information on progesterone alpha vs. beta preference in postmenopausal females.

Oral Progesterone

If progesterone is supplemented orally within 72 hours of sample collection, urine progesterone metabolites are expected to be elevated due to the first-pass effect in the gut and liver. Subsequently, urine concentrations will not correlate with serum concentrations when this route of administration is used.

Orally administered progesterone undergoes several steps of metabolism, including its first steps in the gut when it comes in contact with intestinal bacteria which includes 5b-reductase activity, and then at the gut wall where it has both 5a-reductase activity and conjugation of steroids with glucuronic acid.

As mentioned above, the DUTCH Test measures several of these progesterone metabolites which can offer insights on the role of progesterone on mood and sleep, see **"Progesterone Metabolism" on page 84** for more information.

Progesterone that is within the oral progesterone reference range does not guarantee that the dose and route of administration (ROA) of progesterone is appropriate for endometrial protection when replacement therapy (ERT) is being used concomitantly. Research shows that 100-200 mg of oral progesterone is protective to the endometrium when ERT is being used, however, 200 mg oral progesterone may be more appropriate for moderate to high doses of ERT.

When a female patient reports taking oral progesterone within 72 hours of sample collection, we adjust the progesterone reference ranges on a female report to reflect the a-pregnanediol and b-pregnanediol levels that are typically seen when a standard dose of 100-200 mg is taken during the test (and not skipped).

NOTE

> *The serum equivalent value reported on page 1 of the DUTCH Complete and DUTCH Plus reports is a calculated value from the progesterone metabolites and is not accurate when patients take oral or sublingual progesterone.*

Sublingual Progesterone

If progesterone is supplemented sublingually within 72 hours of sample collection, the portion of the progesterone hormone that is swallowed will elevate urine progesterone metabolites due to the first-pass effect in the gut and liver. Subsequently, like oral progesterone, urine results will not correlate with serum results when sublingual progesterone is taken. We do not have reference ranges for sublingual progesterone due to the high variability in the amount of progesterone that is swallowed.

It is not recommended to use the DUTCH Test results to monitor circulating progesterone levels when sublingual/troche progesterone is being supplemented, but instead to monitor how the progesterone is being metabolized, see **"Progesterone Metabolism" on page 84** for more information.

NOTE

> *The serum equivalent value reported on page 1 of the DUTCH Complete and DUTCH Plus reports is a calculated value from the progesterone metabolites and is not accurate when patients take oral or sublingual progesterone.*

Vaginal Progesterone

Urinary progesterone metabolites may underrepresent circulating progesterone levels, and do not capture endometrial levels. Vaginal progesterone placed in the upper third of the vaginal vault is rapidly absorbed and concentrates higher in the endometrium due to the uterine first-pass effect, thus endometrial progesterone concentrations are often higher than serum progesterone concentrations with this route of administration. Research shows that 45-90 mg of vaginal progesterone may offer endometrial protection when estrogen replacement therapy (ERT) is being used, see **Figure 1.18** for more information.

Transdermal (TD) Progesterone

Transdermal progesterone typically only increases urine progesterone metabolites slightly on the DUTCH Test. Even high doses of TD progesterone do not elevate urinary progesterone metabolites into the luteal range. In general, laboratory monitoring is not helpful for transdermal (TD) progesterone dosing. TD progesterone has not been well studied, and the studies that are available show that it does not circulate well. TD progesterone has not consistently been shown to protect the endometrium when estrogen replacement therapy (ERT) is also being used.

Rectal Progesterone

Urinary progesterone metabolites may be higher than serum results as some of the progesterone may be transported to the liver via the portal vein and become metabolized, though this has not been studied. Rectal progesterone has not demonstrated endometrial protection when estrogen replacement therapy (ERT) is used, and the DUTCH Test results should not be assumed to be useful in assessing for endometrial protection.

PREGNENOLONE THERAPY

Like progesterone, pregnenolone is metabolized into a-pregnanediol and b-pregnanediol. However, unlike progesterone, pregnenolone will not elevate circulating progesterone levels. If pregnenolone is supplemented orally within 72 hours of sample collection, urine progesterone metabolites are expected to be elevated due to the first-pass effect in the gut and liver. Subsequently, urine progesterone results will not correlate with serum results when oral pregnenolone is taken.

Figure 1.18: Oral Micronized Progesterone (OMP) Therapy

For access to our references and additional information about OMP, scan the QR code or visit (https://dutchtest.com/research/).

TESTOSTERONE THERAPY

Using DUTCH for Testosterone Therapy

The best uses of the DUTCH Test with testosterone therapy include:

1. As a compliment to serum for patients on testosterone therapy.
2. Evaluating androgen metabolism pathways. The alpha androgen metabolites (5a-DHT, 5a-androstanediol and androsterone) best reflect androgen activity at the tissue level, whereas the beta androgen metabolites (5b-androstanediol and etiocholanolone) have little to no androgen activity. For example, is testosterone being preferentially metabolized down the more androgenic alpha pathway which could contribute to symptoms of high androgens such as hair loss and irritability? Or is testosterone being preferentially metabolized down the less androgenic beta pathway which could contribute to symptoms of low androgens such as fatigue, low mood, and difficulty losing weight?
3. Monitoring transdermal, intramuscular injection and pellet testosterone therapy, as urine generally correlates with serum. Injections and pellets increase urine testosterone levels, as expected, but the increase may exceed what is seen in serum testing.
4. Evaluating androgen aromatization into estrogens and downstream estrogen metabolism patterns.
5. In men, the addition of epitestosterone as a measured value on the DUTCH test also provides insight into endogenous production of testosterone, as values will reflect (approximately) that of endogenous production of testosterone, and concentrations of epitestosterone are unaffected by administration of exogenous testosterone. This can give you an idea of whether endogenous testosterone production is being suppressed by the testosterone replacement therapy. Based upon our own internal data, if epitestosterone is <10, you can assume there is some testicular suppression and <5 is reflective of complete testicular suppression. In the latter case, you could assume that LH values are very low.

NOTE

Due to the possibility of the UGT deletion (that causes urine testosterone, 5a-DHT and 5b-androstanediol to be falsely low), consider monitoring testosterone replacement therapy (TRT) in the serum and using urine (DUTCH) adjunctively. See **"UGT Deletion" on page 31** *for more information regarding the UGT genetic variant.*

Oral Testosterone

If testosterone is supplemented orally within 72 hours of sample collection, urine testosterone (and downstream metabolites) may be elevated due to the first-pass effect in the gut and liver. Subsequently, urine results may not correlate with serum results when this route of administration is used.

Sublingual Testosterone

If testosterone is supplemented sublingually within 72 hours of sample collection, the portion of the testosterone hormone that is swallowed may elevate urine testosterone (and downstream metabolites) due to the first-pass effect in the gut and liver. Subsequently, like oral testosterone, urine results may not correlate with serum results when sublingual testosterone is taken.

Vaginal Testosterone

There is often the concern that testosterone used vaginally may contaminate the urine and falsely elevate results. DUTCH testing is unique in that free hormones are separated from conjugated hormones prior to analysis allowing for accurate testing even with the use of intravaginal hormones. Both serum and DUTCH results show modest increases in testosterone with doses commonly used (~1mg).

It is recommended to evaluate testosterone as well as the downstream metabolites of testosterone: 5a-DHT, 5a-androstanediol, and 5b-androstanediol. If excessive testosterone replacement therapy (TRT) is used, symptoms of androgen excess (facial hair growth, body hair growth, scalp hair loss, acne, mood changes, etc.) will often manifest.

Transdermal Testosterone

Urinary testosterone levels, as reported on the DUTCH Test, generally parallel changes observed in serum testosterone levels as well as with clinical outcomes (including increased muscle mass, erythrocytosis, etc.) in men.

Testosterone Pellets

Pellets increase the DUTCH Test urinary testosterone levels, as expected, but the increase may exceed what is seen in serum testing. If on a 3-month dosing schedule, consider testing halfway through dosing interval (6-8 weeks after insertion) and baseline trough levels (3-4 months after pellet insertion).

We recommend that DUTCH testing be completed about halfway through the dosing window (so 3 months after pellet insertion if pellets are inserted every 6 months), or 3-4 days after a weekly injection.

Intramuscular Testosterone Injections

Intramuscular (IM) testosterone injections increase the DUTCH Test urinary testosterone levels, as expected, but the increase may exceed what is seen in serum testing. Consider testing halfway through the dosing interval.

Rectal Testosterone

The DUTCH Test urine results may be higher than serum results, as some testosterone may be transported to the liver via the portal vein and undergo first-pass metabolism.

DHEA THERAPY

Using DUTCH for DHEA Therapy

The best uses of the DUTCH Test with DHEA therapy include:

1. Evaluating androgen metabolism pathways: the alpha androgen metabolites (5a-DHT, 5a-androstanediol and androsterone) best reflect androgen activity at the tissue level, whereas the beta androgen metabolites (5b-androstanediol and etiocholanolone) have little to no androgen activity. For example, is DHEA being preferentially metabolized down the less androgenic beta pathway which could contribute to symptoms of low androgens such as fatigue, low mood, and difficulty losing weight? Or is DHEA possibly being over-aromatized into estrogen?
2. Evaluating androgen aromatization into estrogens and downstream estrogen metabolism patterns.

NOTE

7-keto DHEA does not increase DHEA or its metabolites on the DUTCH Test.

Oral DHEA

If DHEA is supplemented orally within 48 hours of sample collection, urine DHEA-S (and downstream metabolites) may be elevated due to the first-pass effect in the gut and liver. Subsequently, urine results may not correlate with serum results when this route of administration is used.

Sublingual DHEA

If DHEA is supplemented sublingually within 48 hours of sample collection, the portion of the DHEA hormone that is swallowed may elevate urine DHEA-S (and downstream metabolites) due to the first-pass effect in the gut and liver. Subsequently, like oral DHEA, urine results may not correlate with serum results when sublingual DHEA is taken.

NOTE

Sublingual/troche DHEA has not been extensively researched.

Transdermal DHEA

The DUTCH test measures predominantly DHEA-S, and because transdermal application bypasses the liver (where much of the DHEA is sulfated to DHEA-S), transdermal DHEA may not increase urinary DHEA-S much.

It is recommended to monitor the androgens (like testosterone, 5a-DHT, 5a-androstanediol, androsterone, and etiocholanolone) and the estrogens (estrone, estradiol, estriol, phase 1 estrogen metabolites, and 2-methoxy-E1) that are downstream of DHEA to evaluate how the supplemented DHEA may be affecting the patient's sex hormone profile.

Vaginal DHEA

As vaginal DHEA has been shown to increase downstream metabolites such as testosterone, the DUTCH Test has proven to be an excellent tool for evaluating DHEA's effects with this route of administration.

It is recommended to monitor the androgens (like testosterone, 5a-DHT, 5a-androstanediol, androsterone, and etiocholanolone) and the estrogens (estrone, estradiol, estriol, phase 1 estrogen metabolites, and 2-methoxy-E1) that are downstream of DHEA to evaluate how the supplemented DHEA may be affecting the patient's sex hormone profile.

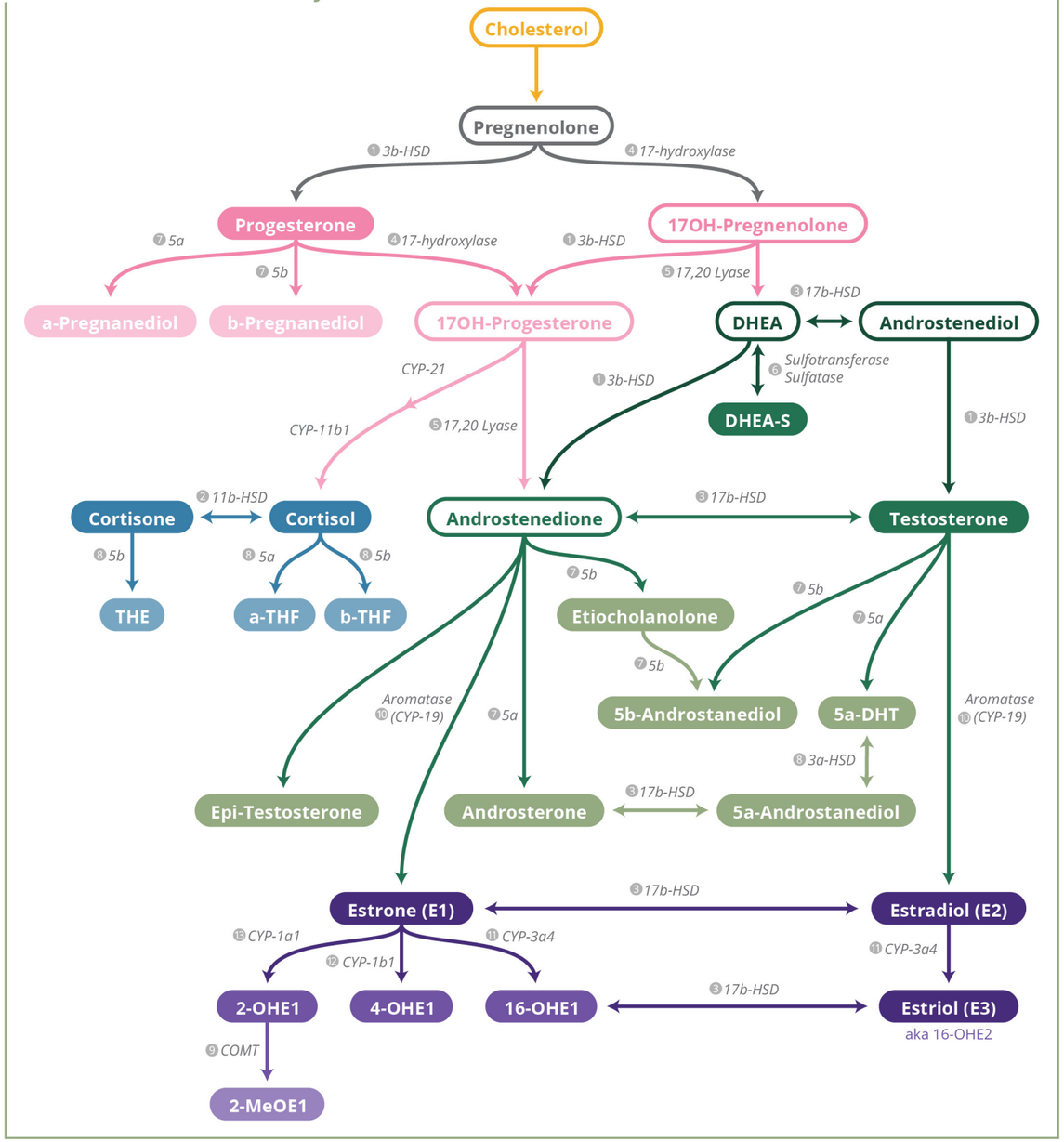


Figure 1.19: ***The DUTCH Steroid Pathway***
Follow the arrows to see how cholesterol is metabolized into the various sex hormones and cortisol.

THE DUTCH STEROID PATHWAY

OVERVIEW

The DUTCH Steroid Pathway is a summary depicting where hormones come from and how they form through various cells in the body. It includes supplements, nutrients, herbs, and medications shown in the literature to increase or decrease particular enzymes affecting these hormones. This is a general steroid pathway and does not specifically differentiate from cells in the ovaries, adrenal glands, or testes.

HOW TO READ THE CHART

Begin at the top of the page, see **Figure 1.19**, with cholesterol and follow the arrows downstream to see the conversion of cholesterol into various steroid hormones. Hormones depicted with a solid color bubble are measured by the DUTCH Test, while hormones depicted with an outline are not. The outlined hormones are too far upstream to test directly with our methodology. Instead, we test the downstream metabolites of that hormone.

Hormones are color-coded for convenient reading:

- Cholesterol
- Pregnenolone
- Cortisol
- Progesterone
- Androgens
- Estrogen

Next to each arrow on the steroid pathway chart is the name of the enzyme responsible for moving each hormone further downstream. These enzymes are important because they can be targeted with lifestyle changes and supplementation to improve symptoms associated hormone imbalances.

CONTRIBUTING FACTORS

Use the corresponding number next to a protein to find the list of contributing factors that may be affecting the results of a DUTCH Test. See **Figure 1.20** for access to our references and additional information about these findings.

1 3b-HSD

May be increased by: Fenugreek, high ACTH/hyperadrenalism, PCOS.

May be decreased by: Isoflavonoids/phytoestrogens, phthalates, organochlorines, BPA, ketoconazole, finasteride, dutasteride.

2 11b-HSD

May push to cortisone: EGCG, PCOS, curcumin, 7-keto-DHEA, progesterone, coffee, holy basil, bitter melon, hyperthyroidism, high estrogens, glucocorticoids.

May push to cortisol: Insulin resistance, obesity, inflammation, hypothyroidism, licorice root, phthalates, organotoxins, alkylphenols, mother's diet during pregnancy.

Figure 1.20: Steroid Pathway Resources & References

For access to our references and additional information, scan the QR code or visit (https://dutchtest.com/steroid-pathway-resources/).

3 17b-HSD

Type 1 (Converts E1 to E2)

May be increased by: High carbohydrate diet

May be decreased by: Flavones, flavanones, isoflavones, genistein, biochanin A, hesperidin, luteolin, tea, citrus, Apiaceous vegetables (carrots, celery, parsley, parsnips, cumin, etc.), red pepper, melatonin, omega-3 fatty acids.

Type 2 (Converts E2 to E1)

May be increased by: Endogenous progesterone, progestins (e.g., in birth control pills).

May be decreased by: Flavones, bioflavonoids, phytoestrogens, rutin, quercetin, myricetin, polyphenols (e.g., Kaempferol)

Type 5 (Converts Androstenedione to Testosterone)

May be increased by: Alcohol

May be decreased by: Coumestrol, biochanin A, quercetin, 18b-glycyrrhetinic acid (licorice), apigenin

NOTE

> *There may be significant overlap in how substances affect the back and forth activity of these enzymes.*

4 17-hydroxylase

May be increased by: Hyperglycemia, hyperinsulinemia.

May be decreased by: Ketoconazole, spironolactone, apigenin, polyphenols.

5 17, 20 Lyase

May be increased by: PCB exposure, DHEA supplements, obesity.

May be decreased by: Licorice root, spironolactone, azole antifungals, hyperglycemia, apegenin.

6 DHEA & DHEA-S

DHEA converts to DHEA-S with hydroxysteroid sulfotransferase while DHEA-S converts to DHEA with steroid sulfatase; these interconvert in different locations within the body.

DHEA-S may be increased by: Spironolactone, dexamethasone, bile acid, St. John's Wort, forskolin.

DHEA-S may be decreased by: Low cysteine, inflammation, LPS, ketoconazole, progestin, licorice.

7 5a-Reductase & 5b-Reductase

5a-Reductase is best known because it makes androgens like testosterone more potent. It is also responsible for metabolizing progesterone and cortisol. If up-regulated, it may cause high androgen symptoms in men (thinning hair, prostate) and women (as in PCOS, thinning hair, acne, facial hair growth). 5b-Metabolites are less androgenic (weaker). 5b-Reductase may be affected by some of the listed things for 5a as well (often to a lesser degree). This same enzyme also metabolizes cortisol, see **"e Cortisol Metabolism/Clearance" on page 49** for more detail.

5a may be increased by: Insulin resistance, obesity, DHEA supplementation, PCOS.

5a may be decreased by: Saw palmetto and beta-sitosterol, reishi, nettle root, pygeum africanum, polyunsaturated fats (PUFAs), and EGCG.

5b may be increased by: Insulin resistance, high triglycerides, PCOS.

5b may be decreased by: Licorice.

8 Cortisol Metabolism/Clearance

Cortisol is metabolized by 5a/5b-reductase (and 3a-HSD) to a/b-THF & THE for excretion. Metabolism may be increased in obesity, high insulin and hyperthyroid. It may be slowed in cases of hypothyroidism, anorexia, cholestasis, or poor liver function. This same enzyme metabolizes testosterone, androstenedione, and progesterone, see **"5a-Reductase & 5b-Reductase" on page 49** for more detail.

9 COMT

May be increased by: S-adenosyl-L-methionine (SAMe), magnesium, choline, B6, B12, folate, betaine/TMG (cofactors).

May be decreased by: Estradiol, phthalate esters, rhodiola rosea, quercetin, catechin, epicatechin.

10 Aromatase (CYP-19)

May be increased by: Obesity and inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony, licorice root, atrazine, rutin.

May be decreased by: Enterolactone, apigenin, genistein, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, aromatase inhibitors (letrozole, anastrozole).

⓫ CYP-3a4

Many common medications induce CYP-3a4, including but not limited to, phenobarbitol, phenytoin, rifampicin, and glucocorticoids.

Many common medications interfere with or competitively inhibit CYP-3a4, including but not limited to, cimetidine, tamoxifen, quinolones, and fluoxetine.

May be increased by: St. John's Wort, pesticides, caffeine, smoking, PAHs, moderate alcohol consumption, obesity.

May be decreased by: Grapefruit, resveratrol, rosemary, wild yam, peppermint oil, azole antifungals.

⓬ CYP-1b1

May be increased by: Inflammation, smoking, PAHs.

May be decreased by: Flavonoids, resveratrol.

⓭ CYP-1a1

May be increased by: Cruciferous vegetables, DIM/IC3, caffeine, soy, fish oil, rosemary extract, thyroxine, flaxseed.

May be decreased by: High sugar diet, moderate alcohol consumption, resveratrol, pterostilbene.

Sex Hormones: Cycling Female

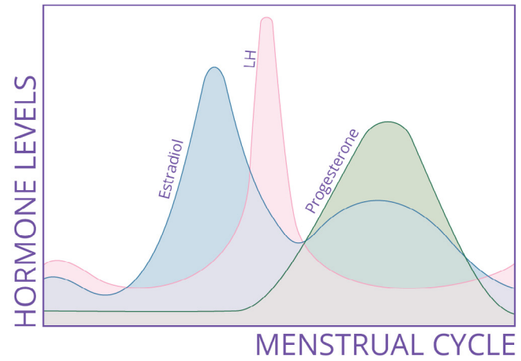


Figure 2.1: ***Hormones Throughout the Menstrual Cycle***
Estrogen and progesterone levels fluctuate significantly during the follicular, ovulatory, and luteal phases of the menstrual cycle.

The Menstrual Cycle

The menstrual cycle is made up of the follicular phase, the ovulatory phase, and the luteal phase, see **Figure 2.1**.

Day one of the menstrual cycle (and day one of the follicular phase) is marked by the onset of menses. During the follicular phase, follicle stimulating hormone (FSH) stimulates multiple follicles to grow within the ovary. While this is happening, luteinizing hormone (LH) stimulates ovarian theca cells to make androgens and FSH stimulates the aromatase enzyme to convert these androgens to estrogens in the ovarian granulosa cells. A single, dominant follicle is selected around cycle days 5-7. This selected follicle will go on to release an egg later in the cycle. The other follicles that were not selected degenerate.

The follicular phase is followed by the ovulatory phase. During the ovulatory phase, surging estradiol released by the dominant follicle signals the pituitary to release a large amount of LH. About 10-12 hours after LH peaks, ovulation occurs, and an egg is released from the follicle. The remaining part of the follicle becomes the corpus luteum, a temporary endocrine structure that produces progesterone through the end of the menstrual cycle. If pregnancy occurs, the corpus luteum will continue to make progesterone until the placenta takes over at the end of the 1st trimester. If there is no pregnancy, then estradiol and progesterone levels decrease, triggering menstrual bleeding and starting the next cycle.

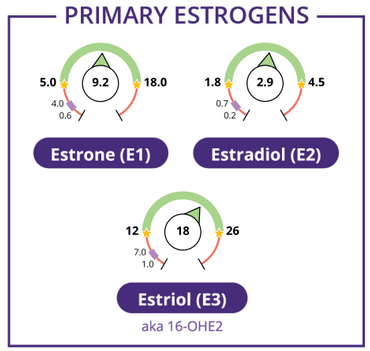
NOTE

A healthy luteal phase is 13-15 days in length and a normal menstrual cycle is typically 24-35 days in length for adults.[d]

[d] Taylor HS, et al. Speroff's Clinical Gynecological Endocrinology and Fertility. Nineth edition. Wolters Klumer; 2020; 137-173.

ESTROGEN

Figure 2.2: Measuring Estrogen



The DUTCH Test measures the three primary estrogens: Estrone, Estradiol, and Estriol.

OVERVIEW

The DUTCH Test measures the three primary estrogens: Estrone (E1), Estradiol (E2), and Estriol (E3), see **Figure 2.2**.

Estradiol has the strongest stimulatory effect on estrogen receptors while estriol has the weakest effect. Estrone and estradiol can interconvert via 17b-HSD and estriol is an estradiol and 16-OH-E1 downstream metabolite.

Estrogen's Role in Cycling Females

In cycling females, estradiol builds the endometrium for possible pregnancy. It is also beneficial for bone health, insulin sensitivity, healthy weight management, neurotransmitter balance, sleep, mood, concentration, memory, hair, skin, vaginal pH and lubrication, and cardiovascular health. Estradiol excess, or estradiol that is not well balanced with progesterone, may have adverse health consequences, which include endometrial cancer, gallstones, clotting issues, heavy bleeding, menstrual cramping, tender or fibrocystic breasts, mood disturbances, acne, fibroids, headaches, and weight gain. See **"Estrogen Dominance Patterns" on page 78** for more information about the progesterone to estrogen balance in cycling females.

Estradiol and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Studies evaluating interactions between the provoked HPA axis and estradiol are limited in both healthy and diseased individuals. The available data on cortisol and estradiol in cycling females states that estradiol can impact the HPA axis, and the HPA axis can impact estradiol levels. With HPA axis dysfunction, the hypothalamic-pituitary-gonadal (HPG) axis may be suppressed, thus decreasing estradiol levels. [e]

Estrogen Production in Cycling Females

In cycling females, estradiol is predominantly made in the ovaries. Estradiol levels are highest during the ovulatory phase and lowest in the early follicular phase.

ESTRADIOL IS LOW

Signs & Symptoms

Low E2 in cycling females may result in hot flashes, night-sweats, insomnia, joint pain, skin issues, low sex drive, mood disturbance, brain fog, vaginal dryness, weight gain, decreased bone mineral density, and increased cardiovascular disease risk.

Potential Root Causes

Low E2 in cycling females is often associated with high stress (HPA axis dysfunction), thyroid disorders, high prolactin, breastfeeding, perimenopause, downregulated aromatase activity[1], improper timing of DUTCH sample collection, e.g., collecting in the first week of the cycle rather than the last half of the cycle[2], medications[3], and diindolylmethane (DIM)/I3C[4]. Less common associations include anorexia, extreme exercise, low body weight, low androgens, decreased ovarian blood flow (i.e., surgery or smoking, and poor ovarian cell health.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other support considerations include herbal and dietary estradiol support, HPA axis support, androgen support if low and promoting regular cycles and ovulation. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

ESTRADIOL IS ELEVATED

Signs & Symptoms

In cycling females, estradiol excess, or estradiol that is not well balanced with progesterone, may have adverse health consequences, which include heavy bleeding, fibroid growth, menstrual cramping, tender or fibrocystic breasts, mood disturbances, fatigue, acne, headaches, weight gain, clotting issues, and gallstones. E2 that is not balanced with Pg may increase endometrial hyperplasia and cancer risk, and elevated endogenous estrogen is a risk factor for breast cancer.

Potential Root Causes

Elevated E2 in cycling females may be normal or may be associated with obesity, insulin resistance, diabetes, inflammation, stress, PCOS, elevated androgens, perimenopause, poor detoxification, endocrine disrupting chemicals (EDCs), high alcohol intake, gut dysbiosis, DHEA/T/E2 supplementation, upregulated aromatase activity[5], or improper timing of DUTCH sample collection, e.g., if samples are collected during the ovulatory E2 surge[6]. Interpret results with caution if oral or sublingual hormones are being supplemented[7].

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other support considerations include supporting estrogen detoxification and healthy metabolism patterns, supporting the HPA axis, lowering inflammation, regulating blood sugar, weight loss (if appropriate), lowering androgens if elevated, and general liver support to further encourage hormone detoxification. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

ESTRADIOL IS WITHIN RANGE

Consider confirming that Pg is within the reference range and balanced with estradiol, especially if the person reports symptoms that are cyclical in nature.

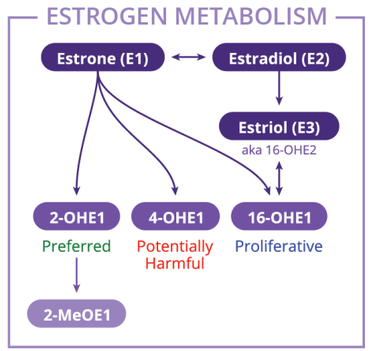
NOTE

Estrogen excess symptoms may have causes other than elevated estradiol relative to progesterone. It is also possible for estradiol to be above the reference range during other times in a female's cycle (outside of the luteal phase measurement period). In this case, a DUTCH Cycle Mapping may be clinically useful. In all cases, ensure healthy phase 1 and phase 2 estrogen metabolism.

[e] Chrousos GP, et al. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med. 1998 Aug 1;129(3):229-40.

ESTROGEN DOMINANCE PATTERNS

Figure 2.3: Estrogen Metabolism



The DUTCH Test measures the three primary estrogens (E1, E2, E3), as well as some important metabolites, including: 2-OH-E1, 4-OH-E1, 16-OH-E1 and 2-MeOE1.

OVERVIEW

The DUTCH Test measures the three primary estrogens, as well as some important metabolites, see **Figure 2.3**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

"Estrogen dominance" is a term used to describe the hormonal imbalance that arises when estrogen levels are relatively higher than progesterone levels in the body. Estrogen dominance can occur when progesterone is low, estrogen is in excess, or when both are within range, but estrogen is relatively higher than progesterone. Overall, when someone is said to be "estrogen dominant," their progesterone to estrogen (Pg/E2) ratio tends to be low. People can become symptomatic if they do not have enough progesterone to balance the estrogen activity in their bodies. While estrogen dominance is most commonly considered in cycling females, it can also occur in peri- and postmenopausal females. See **"Estrogen Dominance Patterns" on page 78** for examples of estrogen dominance in postmenopausal females.

B-pregnanediol/E2 ratio reference ranges for cycling females: 50-300 in the follicular range, <100 during ovulation, and 100-500 in the luteal phase.

Some signs and symptoms of estrogen dominance include:

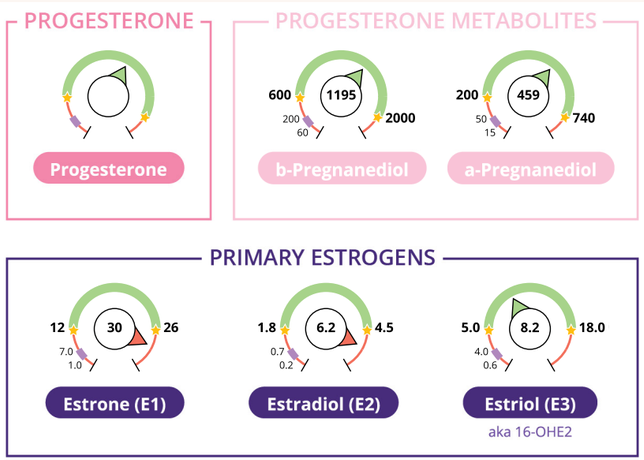
- Heavy bleeding
- Menstrual cramping
- Breast tenderness
- Mood swings
- PMS
- Irritability
- Anxiety
- Sleep issues
- Irregular cycles
- Weight gain
- Acne
- Fertility issues
- Fatigue
- Gallstones
- Headaches
- Clotting issues

PATTERN EXAMPLES

Estrogen dominance may be observed in various patterns on the DUTCH Test, see figures 2.4–2.8 for examples.

Estrogen is Elevated but Progesterone is Not

Estrogen dominance can occur with estrogen excess, see **Figure 2.4**.



Estrogen is Within Range but Progesterone is Below Range

Estrogen dominance can occur with low progesterone, see **Figure 2.5**.

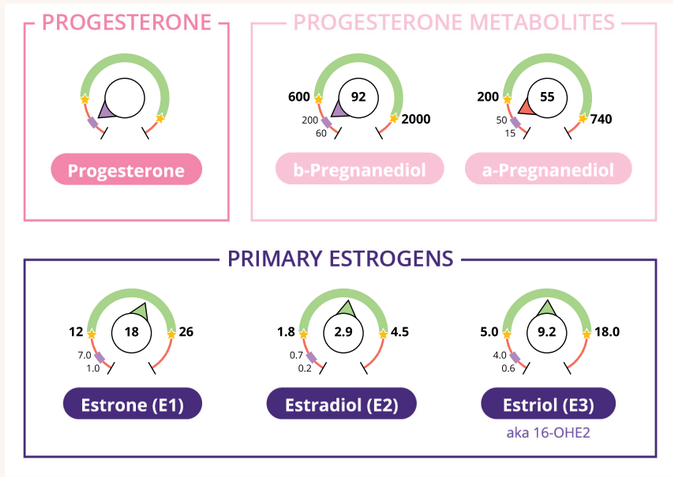


Figure 2.5: *Estrogen is Within Range but Progesterone is Below Range*
Estrogen dominance can occur when progesterone is below range (anovulatory in this example) and estrogen is within range.

Estrogen is Relatively Higher than Progesterone

Estrogen dominance can occur when both estrogen and progesterone are within range, but estrogen is relatively higher than progesterone, see **Figure 2.6**.

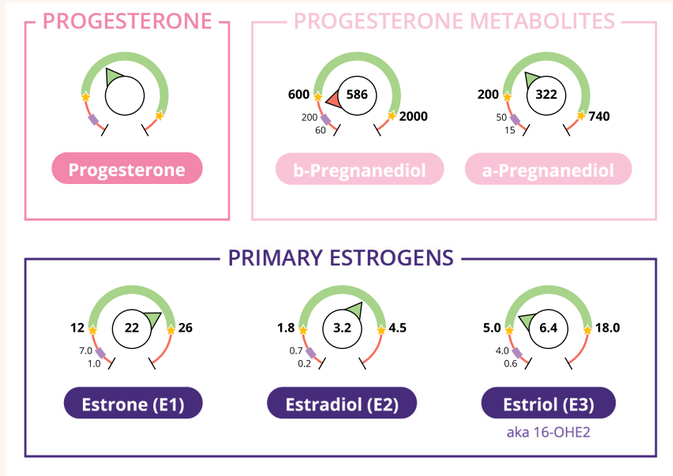


Figure 2.6: *Estrogen is Relatively Higher than Progesterone*
Estrogen dominance can occur when both estrogen and progesterone are within range, but estrogen is relatively higher than progesterone.

Phase 1 Estrogen Metabolism is Elevated

Estrogen dominance can also arise when progesterone and the parent estrogens, estradiol (E2) and estrone (E1), are balanced but phase 1 estrogen metabolites are elevated. This is because the 2-OH, 4-OH, and 16-OH phase 1 estrogen metabolites can actively bind to estrogen receptors, see **Figure 2.7**.

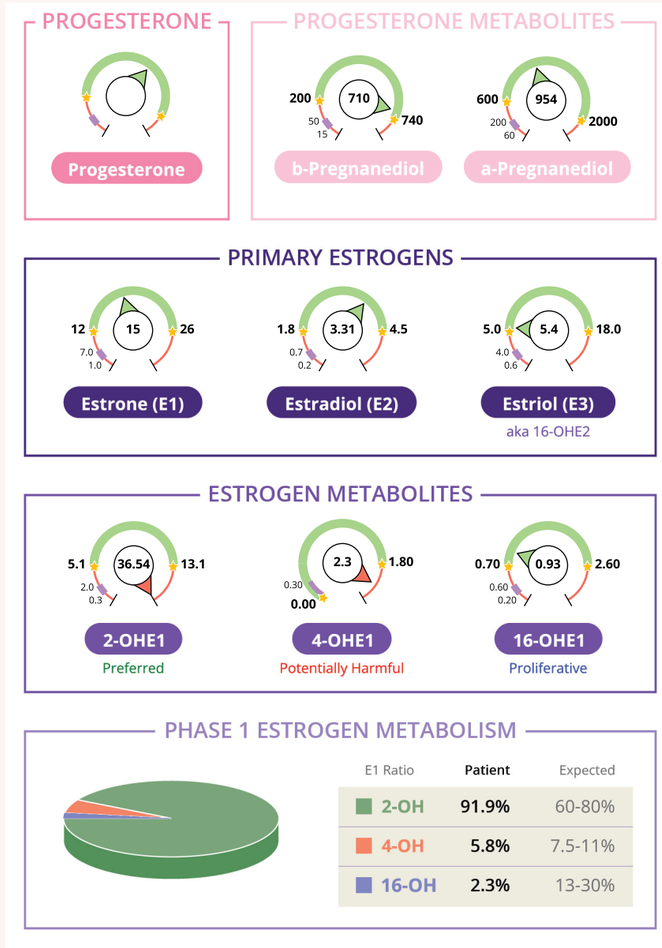


Figure 2.7: ***Phase 1 Estrogen Metabolism is Elevated***
Estrogen dominance can also arise when progesterone and the parent estrogens, estradiol (E2), and estrone (E1) are balanced but phase 1 estrogen metabolites are elevated.

Estrogen is Elevated During Ovulatory Phase

Even if estrogen is well balanced with progesterone during the luteal phase in a cycling woman, estrogen dominance can arise when estrogen surges above range during the ovulatory phase, see **Figure 2.8**.

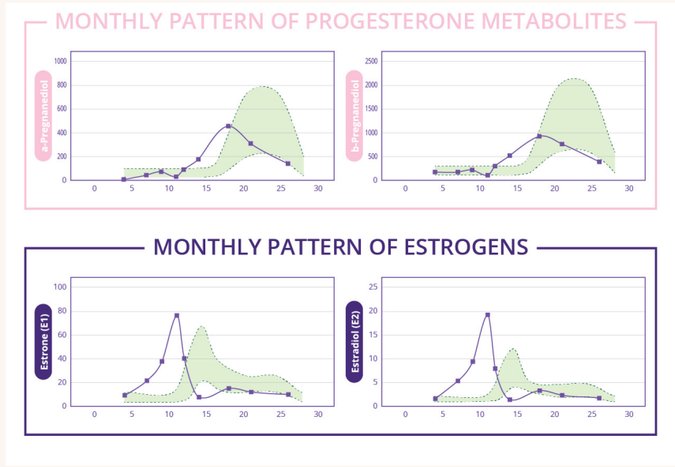
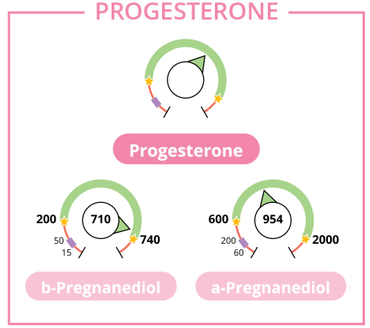


Figure 2.8: ***Estrogen is Elevated During Ovulatory Phase***
Estrogen dominance can arise when estrogen surges above range during the ovulatory phase.

PROGESTERONE

Figure 2.9: Measuring Progesterone



The DUTCH Test measures progesterone (Pg) indirectly by taking the weighted average of two metabolites, a-pregnanediol and b-pregnanediol.

OVERVIEW

The DUTCH Test measures two progesterone metabolites in the urine: a-pregnanediol and b-pregnanediol, see **Figure 2.9**.

a-Pregnanediol modulates GABA receptors in the central nervous system (CNS) and can lead to improvements in mood and sleep. b-Pregnanediol is the major progesterone metabolite, but unlike a-pregnanediol, it does not modulate GABA receptors. These progesterone metabolites in urine strongly correlate to serum progesterone. [f] This is not true when oral or sublingual progesterone is used (see medications section).

Progesterone's Role in Cycling Females

In cycling females, progesterone's primary role is to stabilize the endometrium for conception, but also supports sleep, mood, and memory. In premenopausal and postmenopausal females, progesterone also protects against endometrial cancer, osteoporosis and cardiovascular disease.

Progesterone and the HPA Axis

Studies evaluating interactions between the provoked HPA axis and progesterone are limited in both healthy and diseased individuals. The available data on cortisol and progesterone in cycling females is mixed. However, there is literature documenting that with HPA axis dysfunction, the HPG axis may be suppressed, thus decreasing estradiol and as a result, corpus luteum progesterone. Adrenal progesterone increases in response to stress and increased cortisol output. [g] [h]

Progesterone Production in Cycling Females

In cycling females, progesterone is predominantly made in the ovaries by the corpus luteum during the luteal phase.

PROGESTERONE IS LOW

Signs & Symptoms

Low Pg in cycling females may contribute to fatigue, insomnia, irritability, anxiety, weight gain, infertility, and increase endometrial cancer risk. When Pg is low relative to E2 (relative estrogen dominance), some females may experience PMS, heavy bleeding, and breast tenderness.

[f] Newman M, et al. Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC-MS/MS). BMC Chem. 2019; 13(1): 20.

[g] Chrousos GP, et al. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med. 1998 Aug 1; 129(3):229-40.

[h] Herrera AY, et al. Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol Stress. 2016 Feb 11; 3:96-104.

Potential Root Causes

Low Pg in cycling females is commonly associated with anovulation, stress, breastfeeding, perimenopause, PCOS, thyroid disorders, high prolactin, medications[8], and improper timing of DUTCH sample collection outside the midluteal phase[9].

Less common associations include anorexia, low body weight, extreme exercise, decreased ovarian blood flow (e.g., surgery or smoking), decreased ovarian cell health, premature ovarian insufficiency, hypopituitarism, and hypogonadism.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other considerations include HPO axis support with chasteberry extract, maca, evening primrose oil or borage oil, B6, stress reduction, antioxidant support, or bioidentical Pg. Antioxidant support has also been shown to improve Pg levels by improving ovarian cell health. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

PROGESTERONE IS ELEVATED

Signs & Symptoms

Elevated Pg in cycling females may be normal and asymptomatic or can result in fatigue, increased appetite, irritability, premenstrual dysphoric disorder (PMDD), breast tenderness, and bloating before menses. Slightly elevated progesterone is typically not problematic.

Potential Root Causes

High Pg is often normal, but is also commonly associated with pregnancy, progesterone/pregnenolone supplementation[10], inflammation, obesity, insulin resistance, endocrine disrupting chemicals (EDCs), and stress. Less common associations include congenital adrenal hyperplasia (CAH), nonclassical congenital adrenal hyperplasia (NCAH), and theca cell tumors (rare). With oral or sublingual Pg, high DUTCH Test Pg metabolites (relative to the luteal range) are expected.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other support considerations include reducing inflammation, regulating blood sugar, weight loss (if appropriate), and general liver support to encourage hormone detoxification. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

PROGESTERONE IS WITHIN RANGE

Consider confirming that estradiol is within the luteal range and balanced with progesterone, especially if a person reports symptoms that are cyclical in nature.

ORAL PROGESTERONE REFERENCE RANGES

Oral micronized progesterone (OMP) reference ranges for females:

- a-Pregnanediol: 2,000-9,000 ng/mg
- b-Pregnanediol: 580-3,000 ng/mg

Urinary progesterone metabolite levels strongly correlate with serum progesterone only when progesterone is not being supplemented.

When a person reports taking oral progesterone within 72 hours of sample collection, we adjust the progesterone reference ranges to reflect the a-pregnanediol and b-pregnanediol levels that are typically seen when a standard dose of 100mg is taken during the test and not skipped.

Progesterone that is within the oral progesterone reference range does not guarantee that the dose and route of administration (ROA) of progesterone is appropriate and thus does not guarantee endometrial protection when estrogen is also being supplemented. The primary information that can be gained from DUTCH for patients taking oral progesterone is the relative metabolism of progesterone in the gut. If the patient metabolizes with preference down that 5a-pathway, it is likely that the patient is making large quantities of the sedating, analgesic metabolites like a-pregnanediol and a-pregnanolone, which can act on GABA receptors and help with sleep and mood. Observing the patient's metabolic preference may help optimize treatment or the recommended dose in some cases.

Serum equivalent values reported on page 1 of the DUTCH Complete and DUTCH Plus reports will be higher than reality when progesterone is taken orally or sublingually.

PROGESTERONE METABOLISM

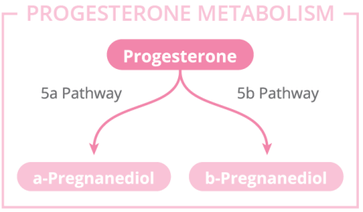
OVERVIEW OF 5-ALPHA VS 5-BETA

The DUTCH Test measures progesterone indirectly by taking the weighted average of a-pregnanediol and b-pregnanediol, see **Figure 2.10**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

Progesterone is metabolized into a-pregnanediol by 5a-reductase and b-pregnanediol by 5b-reductase. It may be helpful to know if there is a 5a or 5b preference because the alpha progesterone metabolites modulate GABA receptors and may help with mood and sleep. When assessing for an alpha or beta preference, compare the direction of the dials rather than the numbers within the dials.

5a-Reductase and 5b-reductase are also involved in androgen (i.e., DHEA and testosterone) and cortisol metabolism. Therefore, the body's overall preference towards alpha or beta metabolism can be further assessed by comparing alpha vs. beta metabolite levels. Alpha metabolites on the DUTCH Test include a-pregnanediol, androsterone, 5a-DHT, 5a-androstanediol, and a-THF. Beta metabolites on the DUTCH Test include b-pregnanediol, etiocholanolone, 5b-androstanediol, b-THF, and b-THE.

Figure 2.10: 5a vs 5b Progesterone Preference



It may be helpful to know if there is a 5a or 5b preference because the alpha progesterone metabolites modulate GABA receptors and may help with mood and sleep.

CLINICAL SIGNIFICANCE

a-Pregnanediol Preference

a-Pregnanediol is a GABA receptor modulator that supports mood and sleep. However, in some cycling females it exerts a paradoxical effect that has been associated with PMDD[11]. There is some evidence that a a-pregnanediol preference may increase breast cancer risk, however, more studies are needed to clarify this relationship.

Increased 5a-reductase activity is associated with inflammation, insulin resistance, obesity, DHEA supplementation, PCOS, and certain genetic makeups. Females supplementing with oral Pg may see greater improvements in mood and sleep if they favor the alpha pathway.

b-Pregnanediol Preference

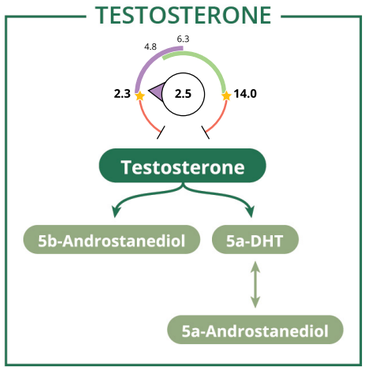
b-Pregnanediol is the major progesterone metabolite, but unlike a-pregnanediol, it does not exert calming effects in the brain through GABA receptor modulation.

5b-Reductase activity is increased by insulin resistance, high triglycerides, PCOS, and certain genetic makeups. People may also favor the beta pathway if they are taking a prescription 5a-blocker (finasteride, dutasteride, etc.)[12] or natural 5a-blockers.

When oral Pg is supplemented, females favoring the beta pathway may need a higher Pg dose than females favoring the alpha pathway to see similar improvements in mood and sleep. In these cases, GABA support may also be considered. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

TESTOSTERONE

Figure 2.11: Measuring Testosterone



The DUTCH Test reflects bioavailable testosterone, as well as it's downstream metabolites: 5a-DHT, 5a-androstanediol, and 5b-androstanediol.

OVERVIEW

The DUTCH Test reflects bioavailable testosterone. Testosterone's activity may be evaluated by looking at its downstream metabolites, 5a-DHT, 5a-androstanediol, and 5b-androstanediol, see **Figure 2.11**.

In target tissues, testosterone is activated to 5a-DHT by the enzyme 5a-reductase. 5a-DHT is three times more potent than testosterone and is made and metabolized locally in the tissues to 5a-androstanediol. Therefore, urinary 5a-androstanediol may be a better marker of tissue 5a-DHT activity than urinary 5a-DHT. In addition to the 5a-DHT and 5a-androstanediol, the downstream alpha metabolite of DHEA (androsterone) can additionally be used to evaluate tissue androgen activity.

NOTE

Some 5a-DHT and 5a-androstanediol can also be formed from DHEA. Thus, if 5a-DHT and/or 5a-androstanediol are elevated, it may be helpful to compare total DHEA and testosterone to better understand where the excess androgens are originating from (the adrenals or the ovaries). In females, if DHEA-S or total DHEA is high, the source of excess androgens is likely the adrenal glands. Conversely, if these are within range, but testosterone and its metabolites are high, the source may be ovarian.

Testosterone can also be metabolized down the less androgenic beta pathway. The enzyme 5b-reductase is concentrated in the liver (as opposed to target tissues) and converts testosterone into 5b-androstanediol. This testosterone metabolite does not correlate with classic androgen symptoms such as body hair, libido, muscle mass, and acne due to the low androgenic potential of beta metabolites.

NOTE

Certain genetic profiles can cause testosterone to be falsely low in the urine, see **"UGT Deletion" on page 31** *for more information.*

Testosterone's Role in Cycling Females

In cycling females, testosterone supports muscle, strength, stamina, healthy weight, bone, skin, hair, mood, memory, sexual function, fertility, cardiovascular health, and estradiol levels (though it contributes to estradiol levels more in postmenopausal females).

Testosterone Production in Cycling Females

In cycling females, testosterone is made in the ovaries (about 25%) and the adrenals (about 25%), and the remaining 50% is aromatized in peripheral tissues (primarily fat) from androstenedione and DHEA. Testosterone production peaks in the third and fourth decades of life (20s and 30s) and declines thereafter.

Testosterone age-dependent reference ranges for females:

- 20-39 years old: 3.2-14 ng/mg
- 40-60 years old: 2.3-8 ng/mg
- > 60 years old: 1.5-6.3 ng/mg

TESTOSTERONE IS LOW

NOTE

Individuals with a UGT deletion (genetic variant) may have falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** *for more information on this genetic deletion and the impact on DUTCH testing.*

Signs & Symptoms

Low testosterone in cycling females may be associated with fatigue, weight gain, difficulty building muscle mass, bone loss, mood issues, brain fog, low libido, and sexual dysfunction.

Potential Root Causes

Low testosterone in cycling females may be associated with aging, elevated sex hormone binding globulin (SHBG), low adrenal output (HPA axis dysfunction), poor ovarian production, surgically removed ovaries, diabetes, hypothyroidism, sleep disturbances, endocrine disrupting chemicals (EDCs), zinc deficiencies, certain medications[13], and upregulated aromatase activity[14].

Low testosterone in females is less commonly associated with traumatic brain injuries, prolactinoma, extremely low body fat percentage, extremely low calorie intake, stress, overexercising, low cholesterol (LDL < 40mg/dL), and suboptimal mitochondrial function.

Potential Support Considerations

In addition to treating the underlying cause, obtain serum total, free, and bioavailable testosterone levels and if low consider zinc, herbal testosterone support, HPA axis support, blood sugar regulation, improving sleep, DHEA[15] and/or testosterone replacement therapy[16]. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

If E1 and E2 are elevated, consider natural aromatase inhibition[17].

TESTOSTERONE IS ELEVATED

Signs & Symptoms

Elevated testosterone in cycling females has been associated with acne, oily skin, increased body/facial hair, thinning scalp hair, androgenic alopecia, and mood disturbance (aggression, irritability, etc.).

Mildly elevated androgens that are not accompanied with symptoms of androgen excess may be normal for some women.

Potential Root Causes

Elevated testosterone cycling in females may be associated with PCOS, low SHBG, downregulated aromatase activity[18], stress, obesity, blood sugar dysregulation,

inflammation, testosterone and/or DHEA supplementation[19], transference (from a partner, the gym, etc.), medications[20], or sample contamination[21].

Less common associations include thyroid issues, NCAH, virilizing tumors, and elevated growth hormone (GH).

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing inflammation, regulating blood sugar, weight loss if appropriate, supporting the HPA axis, supporting liver detoxification, flaxseeds, myo-inositol, herbal anti-androgens, and medications such as metformin and spironolactone, if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

TESTOSTERONE IS WITHIN RANGE

When testosterone is within range, take the alpha androgen levels (5a-DHT, 5a-androstanediol, and androsterone) into consideration, as these also have androgenic actions in the body. If there are androgen excess symptoms, but all these androgens are within range or low, consider other causes for these symptoms, such as hypothyroidism, stress, autoimmune issues, mineral deficiencies, etc.

Always confirm DUTCH results with serum testosterone before treatment, as the UGT genetic variant can result in falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** for more information on this genetic deletion and the impact on DUTCH testing.

OVERVIEW

The DUTCH Test measures three DHEA metabolites: DHEA-S, etiocholanolone, and androsterone, see **Figure 2.12**.

DHEA is considered a pro-hormone, as most is converted at the tissue level to more potent androgens and estrogens. DHEA in the adrenal gland is sulfated to DHEA-S, a form that is more stable in serum and can act as a DHEA reserve. The best way to assess the total adrenal androgen production is to add up the DHEA-S, etiocholanolone, and androsterone metabolites, to equal the total DHEA production, also known as the total adrenal androgen production. It is our experience that total DHEA production may correlate most strongly with serum DHEA-S.

NOTE

Total DHEA = DHEA-S + etiocholanolone + androsterone

DHEA's Role in Cycling Females

DHEA supports muscle, bone, sexual function, fertility, brain health, immune function, and cardiovascular health.

DHEA's Production in Cycling Females

In cycling females, DHEA is primarily made in the adrenal zona reticularis (about 80%) and the rest is made in the ovaries (about 10%) and brain (about 10%). 100% of DHEA-S is made in the adrenals. DHEA production peaks in young adulthood (20s) and then declines with age.

Total DHEA age-dependent reference ranges for females:

- 20-39 years old: 1300-3000ng/mg
- 40-60 years old: 750-2000ng/mg
- > 60 years old: 500-1200ng/mg

DHEA IS LOW

Signs & Symptoms

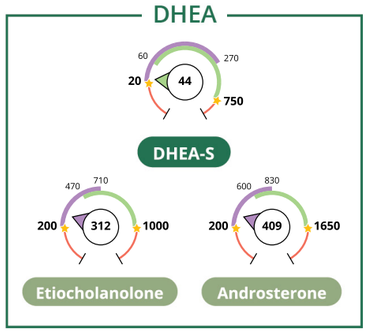
Low DHEA in cycling females may be associated with fatigue, low libido, depression, infertility, diabetes, heart disease, inflammation, and immune disorders.

Potential Root Causes

Low DHEA in cycling females may be associated with older age, decreased adrenal synthesis (HPA axis dysfunction) secondary to chronic stress, inflammation and inflammatory syndromes, blood sugar dysregulation, hypothyroidism, and medications such as glucocorticosteroids, opioids, budesonide inhalers, and metformin.

Higher DHEA-S relative to lower total DHEA may be associated with upregulated SULT2A1 activity (which sulfates DHEA) or a lower STS enzyme activity (removes the "S" from DHEA-S).

Figure 2.12: Measuring DHEA



The DUTCH Test measures three DHEA metabolites including: DHEA-S, etiocholanolone, and androsterone.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include DHEA replacement therapy[22], addressing chronic inflammation, regulating blood sugar, supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, parasympathetic nervous system (PNS) support, and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

DHEA IS ELEVATED

Signs & Symptoms

Elevated DHEA in cycling females has been associated with androgen excess symptoms including oily skin, acne, sleep problems, headaches, and mood disturbances.

Potential Root Causes

Elevated DHEA in cycling females may be associated with DHEA supplementation[23], stress, high adrenal cortisol output (HPA axis dysfunction), inflammation, blood sugar dysregulation, alcohol, nicotine, PCOS, testosterone supplementation[24], and medications such as Alprazolam, Anastrozole, Methylphenidate, Amlodipine, Diltiazem, and Bupropion.

Less common associations include NCAH, hyperprolactinemia, and an adrenal tumor.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing chronic inflammation, regulating blood sugar, supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, PNS support, and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

OVERVIEW OF 5-ALPHA VS 5-BETA

The DUTCH Test measures bioavailable testosterone, DHEA-S and their metabolites, see **Figure 2.13**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

Regarding androgen metabolism, a person may favor the more androgenic alpha pathway, the less androgenic beta pathway, or a balance between the two pathways.

5a-Reductase converts DHEA and testosterone into the alpha androgen metabolites: androsterone, 5a-DHT, and 5a-androstanediol. 5a-DHT is testosterone's active metabolite. It is three times more potent than testosterone, and if elevated may contribute to androgen excess symptoms. 5a-DHT is a peripheral hormone, and research shows that 5a-androstanediol may be a better marker of 5a-DHT tissue activity. [i]

5b-Reductase converts DHEA and testosterone into the beta androgen metabolites: etiocholanolone and 5b-androstanediol. The beta androgen metabolites are generally less androgenic than the alpha androgen metabolites and are formed in the liver, not the tissues. Therefore, these beta metabolites are less likely to contribute to androgen excess symptoms. For example, a person who has elevated testosterone may not experience androgen excess symptoms if they metabolize most of their testosterone down the less androgenic beta pathway.

NOTE

The slider on the DUTCH Test, see **Figure 2.14**, *shows the relative ratio of 5a to 5b metabolites but does not express the absolute value of 5a-DHT and 5a-androstanediol, or if 5a-reductase inhibition is or is not indicated. Consider symptoms and look at the 5a-DHT, androsterone and 5a-androstanediol results if high androgen symptoms are a concern.*

5a-Reductase and 5b-reductase are also involved in androgen (i.e., DHEA and testosterone) and cortisol metabolism. Therefore, the body's overall preference towards alpha or beta metabolism can be further assessed by comparing alpha vs. beta metabolite levels. Alpha metabolites on the DUTCH Test include a-pregnanediol, androsterone, 5a-DHT, 5a-androstanediol, and a-THF. Beta metabolites on the DUTCH Test include b-pregnanediol, etiocholanolone, 5b-androstanediol, b-THF, and b-THE.

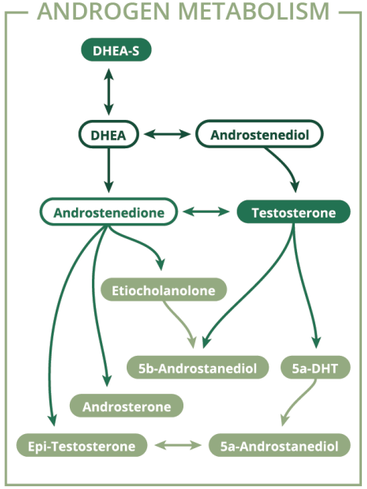
CLINICAL SIGNIFICANCE

5a-Reductase Preference

A metabolic preference for the alpha pathway may lead to elevated androsterone, 5a-DHT, 5a-androstanediol, and excess androgen symptoms. Inflammation, high

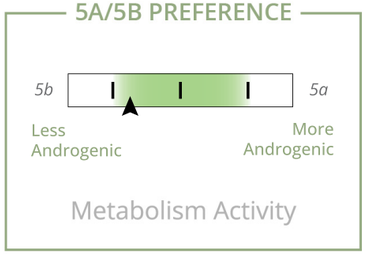
[i] Trabert B, et al. Progesterone and Breast Cancer. Endocr Rev. 2020 Apr 1; 41(2):320–44.

Figure 2.13: Androgen Metabolism



The DUTCH Test measures several androgenic metabolites that stem from testosterone and DHEA, to provide a more complete picture of a patients' metabolic health.

Figure 2.14: 5a vs 5b Androgen Preference



It may be helpful to know if there is a 5a or 5b androgen preference because a beta preference may exacerbate signs and symptoms of low androgens (e.g., low libido, fatigue, weight gain), while an alpha preference may exacerbate signs and symptoms of high androgens (e.g., scalp hair loss, acne, body hair growth)

stress lifestyles, obesity, and blood sugar dysregulation can upregulate 5a-reductase. Alpha preference is often seen with PCOS.

Consider addressing these root causes if the goal is to decrease the alpha preference. Also consider 5a-reductase herbal blockers and medications such as metformin, spironolactone, or finasteride[25], if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

If DHEA and testosterone are low, a metabolic preference for the 5a-reductase enzyme may not necessarily lead to elevated 5a-DHT levels and symptoms of androgen excess.

5b-Reductase Preference

A metabolic preference for the beta pathway may lead to low androsterone, 5a-DHT, 5a-androstanediol, and low androgen symptoms.

Certain genetic makeups, 5a-reductase herbal blockers, and medications such as spironolactone and finasteride[26] may contribute to a beta preference, so consider addressing these root causes if the goal is to decrease the beta preference. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Consider upregulating 5a-reductase activity with high intensity interval training (HIIT), weight resistance exercises, and supplements such as forskolin and pine pollen. Low androgen symptoms associated with low DHEA and/or testosterone may improve by directly addressing DHEA and testosterone levels.

NOTE

If DHEA and testosterone are elevated, a metabolic preference for the beta pathway may be beneficial, as it may help to keep 5a-DHT within range.

ANNOTATIONS

1 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

2 Ensure the person collected the samples at the correct time in their cycle, as the DUTCH Test estradiol reference ranges reflect midluteal levels. For proper evaluation of estrogen and progesterone in the luteal phase, it is best if the first day of menses occurs 4-10 days after collection. Ideally, a female will collect 5-7 days after ovulation, or on cycle days 19-22 for a 28-day cycle. If the person did not collect during the midluteal phase, look at "Additional Normal Ranges" on page 2 of the DUTCH Complete and DUTCH Plus, or page 1 of the DUTCH Sex Hormones panel when interpreting the results.

3 Medications that can contribute to low estradiol include some forms of hormonal birth control (pill, patch, ring, implant, injection), progestins, gonadotropin-releasing hormone agonists, opioid pain medications, glucocorticoids, and NSAIDS.

4 DIM and indole-3-carbinol (I3C) are sometimes used to induce CYP-1a1 (the preferred pathway towards 2-OH), however be mindful that they also tend to lower E1 and E2 which may not be appropriate for every person. When using DIM and/or I3C, it is important that phase 2 detoxification is well supported so that the phase 1 metabolites do not build up in the body and increase propensity for oxidative damage. If on estrogen replacement therapy (ERT), a relatively higher dose of estrogen may be needed when used in combination with estrogen-lowering compounds such as DIM and/or I3C to achieve desired clinical outcomes. If on Tamoxifen, note that DIM and I3C may render Tamoxifen less effective.

5 Aromatase activity may be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

6 Ensure the person collected the samples at the correct time in their cycle, as the DUTCH Test estradiol reference ranges reflect midluteal levels. For proper evaluation of estrogen and progesterone in the luteal phase, it is best if the first day of menses occurs 4-10 days after collection. Ideally, a female will collect 5-7 days after ovulation, or on cycle days 19-22 for a 28-day cycle. If the person did not collect during the midluteal phase, look at "Additional Normal Ranges" on page 2 of the DUTCH Complete and DUTCH Plus, or page 1 of the DUTCH Sex Hormones panel when interpreting the results.

7 Use caution when interpreting estrogen results if estrogen was supplemented orally or sublingually, as the portion that is swallowed undergoes first-pass metabolism and results in elevated urinary estrogen metabolites that do not correlate with serum levels. We suggest stopping oral estrogen 72 hours prior to collection to avoid false elevations in estrogen metabolites. Note that high doses of oral or sublingual DHEA and testosterone may also lead to elevated urinary estrogen metabolites. It may be suggested to stop oral DHEA 48 hours and testosterone 72 hours prior to sample collection.

8 Medications that can contribute to low Pg include some forms of hormone birth control (pill, patch, ring, implant, injection), progestins, gonadotropin-releasing hormone agonists, some fertility medications, opioid pain medications, glucocorticoids, and NSAIDS.

9 Ensure the person collected the samples at the correct time in their cycle, as DUTCH progesterone reference ranges reflect midluteal levels. For proper evaluation of estrogen and progesterone in the luteal phase, it is best if the first day of menses occurs 4-10 days after collection. Ideally, a female will collect 5-7 days after ovulation, or on cycle days 19-22 for a 28-day cycle. If the person did not collect during the midluteal phase, look at the additional reference ranges on page 2 of the DUTCH Complete and DUTCH Plus reports, or on page 1 of the DUTCH Sex Hormones panel when interpreting the results.

10 Urinary progesterone metabolite levels strongly correlate with serum progesterone only when progesterone is not being supplemented. Use caution when interpreting progesterone results if progesterone or pregnenolone was supplemented orally or sublingually within 72 hours of sample collection, as the portion that is swallowed undergoes first-pass metabolism and results in elevated urinary metabolites that do not correlate with serum levels. See "Oral Progesterone Reference Ranges" section above for more information. Note that we do not have reference ranges for sublingual progesterone due to the high variability in the amount of progesterone that is swallowed.

11 In some females, the alpha progesterone metabolites exert a paradoxical effect on the GABA receptors and instead of promoting a sense of calm, they promote anxiety and irritability. This paradoxical effect has been associated with PMDD. Females prone to the paradoxical effect have the most reduction in symptoms when the alpha progesterone metabolites are low (follicular phase levels) or high (above luteal phase levels) and tend to be most symptomatic during their luteal phase. The

pharmaceutical 5a-reductase inhibitor dutasteride has been shown to significantly improve PMDD symptoms, and some persons see benefits from natural 5a-reductase blockers (saw palmetto/beta-sitosterol, reishi, nettle root, epigallocatechin gallate (EGCG), and Pygeum africanum) due to their effect on lowering the alpha progesterone metabolite levels. Oppositely, increasing progesterone with oral micronized progesterone may increase alpha progesterone metabolites (including allopregnanolone) so much that the GABA receptors become calming again. Note that practitioners may choose to use 5a-reductase blockers OR oral progesterone to impact PMDD symptoms, but usually do not use both at the same time.

12 In males and females, the 5a-reductase inhibitors, finasteride and dutasteride, may cause irreversible sexual dysfunction and infertility.

13 Medications that may contribute to low testosterone include glucocorticosteroids, opioids, Accutane, spironolactone, and some oral contraceptive pills.

14 Aromatase activity may be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

15 Use caution with oral DHEA supplementation as it may elevate estrogen.

16 Testosterone should always be evaluated and confirmed in the serum before initiating testosterone replacement therapy (TRT) for low testosterone.

17 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

18 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

19 Use caution when interpreting testosterone results if oral or sublingual DHEA or testosterone were supplemented, as these undergo first-pass metabolism which results in elevated urinary metabolites that do not correlate with serum levels.

20 Medications that may contribute to elevated testosterone include aromatase inhibitors, clomiphene citrate (a selective estrogen receptor modulator (SERM)), and luteinizing hormone (LH), growth hormone (GH), and gonadotropin-releasing hormone (GnRH) analogs (hCG, gonadorelin, kisspeptin, etc.).

21 If testosterone is elevated but the downstream androgen metabolites are not (5a-DHT, androsterone, 5a-androstanediol, etiocholanolone, and 5b-androstanediol) consider the possibility of testosterone sample contamination from someone using TRT (testosterone replacement therapy).

22 Use caution with oral DHEA supplementation, as it may elevate estrogens in addition to increasing testosterone.

23 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into and elevate androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Consider stopping oral DHEA 48 hours prior to testing and oral testosterone 72 hours prior to testing to avoid false elevations.

24 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into and elevate androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Consider stopping oral DHEA 48 hours prior to testing and oral testosterone 72 hours prior to testing to avoid false elevations.

25 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

26 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

Sex Hormones: Postmenopausal Female

Introduction

Postmenopausal females are the most common type of patient that providers order DUTCH tests for, often as a baseline measure or used in conjunction with monitoring various forms of hormone replacement therapy (HRT).

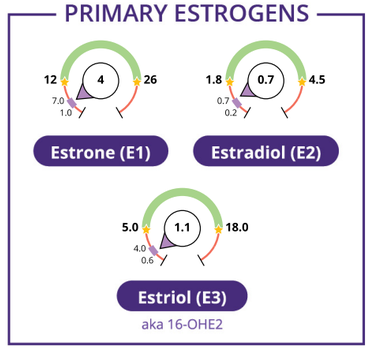
You can gain a lot of knowledge about postmenopausal women from the DUTCH Test. Some of the common reasons for ordering include understanding hormonal symptoms, ensuring that estrogen metabolism is efficient before starting estrogen replacement therapy (ERT), evaluation of symptoms of estrogen dominance, and monitoring therapy to ensure that the patient is in an ideal range.

In this section of the guide, we will share with you the most essential information that you need to interpret your postmenopausal female patients' results. This includes how to interpret each section of their reproductive hormone pages. To understand their HPA axis function, please refer to the separate section in this guide on cortisol and the HPA axis.

LET'S GET STARTED ▶▶

ESTROGEN

Figure 3.1: Measuring Estrogen



The DUTCH Test measures the three primary estrogens: Estrone, Estradiol, and Estriol.

OVERVIEW

The DUTCH Test measures the three primary estrogens: Estrone (E1), Estradiol (E2), and Estriol (E3), see **Figure 3.1**.

Estradiol has the strongest stimulatory effect on estrogen receptors while estriol has the weakest effect. Estrone and estradiol can interconvert via 17b-hydroxy steroid dehydrogenase (HSD) and estriol is an estradiol and 16-OH-E1 downstream metabolite.

Estrogen's Role in Postmenopausal Females

In postmenopausal females, estradiol is beneficial for bone health, insulin sensitivity, healthy weight management, neurotransmitter balance, sleep, mood, concentration, memory, hair, skin, vaginal pH and lubrication, and cardiovascular health. Estradiol excess, or estradiol that is not well balanced with progesterone, may have adverse health consequences, which includes endometrial cancer, gallstones, clotting issues, breast tenderness, and abnormal uterine bleeding (AUB). See **"Estrogen Dominance Patterns" on page 78** for more information about the progesterone to estrogen balance in postmenopausal females.

Estrogen Production in Postmenopausal Females

In postmenopausal (PMP) females, estrone is the predominant estrogen. Estradiol and estrone are primarily synthesized in fat tissue from adrenal androgen aromatization, e.g., androstenedione to estrone and testosterone to estradiol.

ESTRADIOL IS LOW

Signs & Symptoms

Low E2 in PMP females may result in hot flashes, night-sweats, insomnia, joint pain, skin issues, low sex drive, mood issues, brain fog, vaginal dryness, weight gain, decreased bone mineral density, and increased cardiovascular disease risk.

Potential Root Causes

In PMP females, E2 is made from adrenal androgens, thus low E2 can be associated with low adrenal DHEA and androstenedione production, and downregulated aromatase activity[27]. It is plausible that low E2 may occur secondary to extremely low body fat percentages, low cholesterol (LDL < 40mg/dL), poor mitochondrial function, or diindolylmethane (DIM)/I3C[28].

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other considerations include herbal and dietary E2 support, E2 replacement therapy (ERT)[29], androgen support if low, and HPA axis support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

ESTRADIOL IS ELEVATED

Signs & Symptoms

PMP females not on menopausal hormone therapy (MHT), with E2 and estrogen metabolite levels higher than the menopausal reference range, but below a cycling female's luteal range, may still experience low E2 symptoms (see low E2 symptoms above).

PMP females on estrogen replacement therapy (ERT) may experience high E2 symptoms (estrogen dominant symptoms), e.g., AUB, fibroid growth, tender breasts, weight gain, acne, headaches, mood disturbances, swelling, clotting issues, and gallstones.

PMP females with a uterus who are prescribed MHT and have high E2 levels that are not balanced with an appropriate Pg dose may be at increased risk for endometrial cancer.

Potential Root Causes

High E2 in PMP females not on ERT may be normal or associated with elevated androgens, obesity, blood sugar dysregulation, inflammation, or upregulated aromatase activity[30]. In PMP females prescribed DHEA and/or testosterone (T), these hormones may be metabolized to E1 and/or E2, respectively. ERT may also elevate systemic E2 levels, but this is dependent on the dose and ROA.

NOTE

Interpret results with caution if oral or sublingual DHEA, testosterone, E2 is being supplemented[31].

Potential Support Considerations

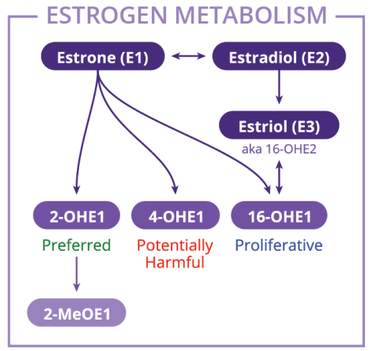
In addition to assessing and treating the underlying root cause, other considerations include supporting estrogen detoxification and healthy metabolism patterns supporting the HPA axis, lowering inflammation, regulating blood sugar, weight loss (if appropriate), lowering androgens if elevated, and general liver support to further encourage hormone detoxification. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

ESTRADIOL IS WITHIN RANGE

ERT[32] may still be appropriate on a case-by-case basis even when E2 is within the normal PMP range. E2 improves bone mineral density, menopausal symptoms, and decreases cardiovascular mortality.

ESTROGEN DOMINANCE PATTERNS

Figure 3.2: Estrogen Metabolism



The DUTCH Test measures the three primary estrogens (E1, E2, E3), as well as some important metabolites, including: 2-OH-E1, 4-OH-E1, 16-OH-E1 and 2-MeOE1.

OVERVIEW

The DUTCH Test measures the three primary estrogens, as well as some important metabolites, see **Figure 3.2**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

"Estrogen dominance" is a term used to describe the hormonal imbalance that arises when estrogen levels are relatively higher than progesterone levels in the body. Estrogen dominance can occur in a postmenpausal female when estrogen is in excess, phase 1 estrogen metabolites are elevated, or when a female uses estrogen replacement therapy (ERT) without (or inadequate) progesterone therapy. Overall, the progesterone to estrogen (Pg/E2) ratio tends to be low. People can become symptomatic if they do not have enough progesterone to balance the estrogen activity in their bodies.

Some signs and symptoms of estrogen dominance in PMP females include:

- Endometrial hyperplasia
- Abnormal uterine bleeding (AUB)
- Possible fibroid growth
- Breast tenderness
- Mood issues
- Weight gain
- Acne
- Fatigue

PATTERN EXAMPLES

Estrogen is Relatively Higher than Progesterone

Estrogen dominance can occur when estrogen is relatively higher than progesterone, see **Figure 3.3**. Additionally, if all three phase 1 estrogen metabolites are pointing above the PMP range and into the luteal range, it creates a pattern that tends to be seen in postmenopausal females who are overweight with high androgens.

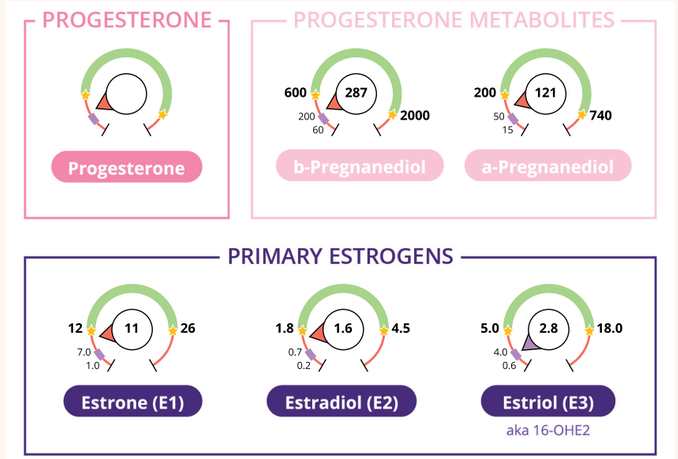


Figure 3.3: *Estrogen is Relatively Higher than Progesterone*
Estrogen dominance can occur when estrogen is relatively higher than progesterone.

Phase 1 Estrogen Metabolism is Elevated

Estrogen dominance can occur when estrogen (E1, E2) and progesterone are within the PMP range, but phase 1 estrogen metabolites are above the PMP range, see **Figure 3.4**. This is because the 2-OH, 4-OH and 16-OH phase 1 estrogen metabolites can actively bind to estrogen receptors.

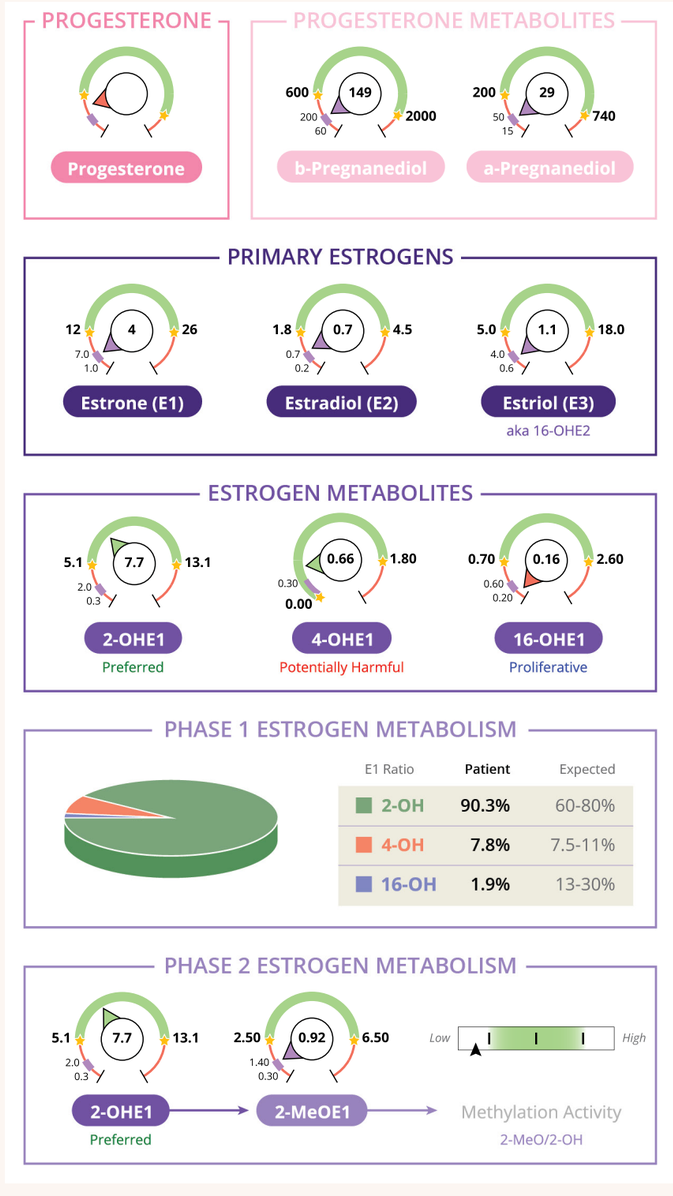


Figure 3.4: *Phase 1 Estrogen Metabolism is Elevated*
E1, E2 and progesterone are within the postmenopausal reference range, but the 4-OH-E1 and 2-OH-E1 phase 1 estrogen metabolites are above range, likely due to low methylation.

Estrogen Replacement Therapy without Progesterone

Estrogen dominance may be observed when estrogen replacement therapy (ERT) is used without (or with inadequate) progesterone therapy. In **Figure 3.5**, the patient was taking a moderately high dose of estradiol (E2) and no progesterone therapy.

NOTE

> *When a female has a uterus, ERT must be used in combination with progesterone to protect the endometrium from hyperplasia and cancer.*

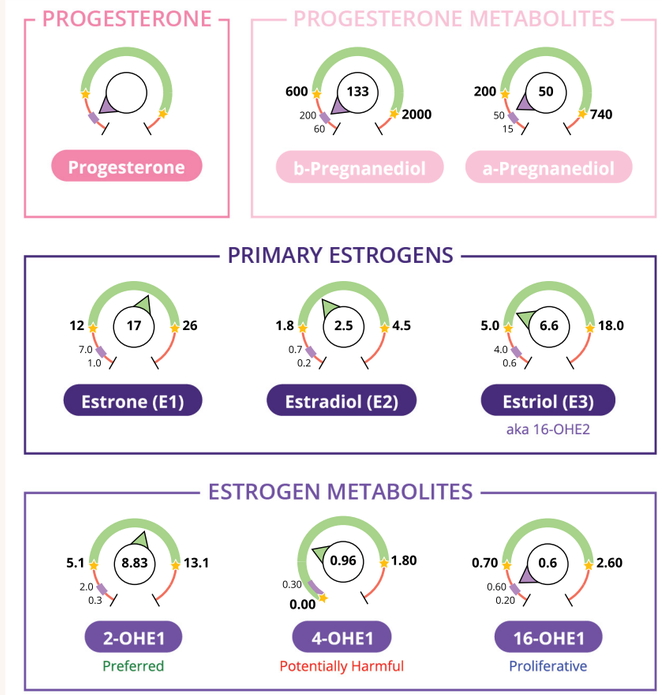


Figure 3.5: ***Estrogen Replacement Therapy without Progesterone***
Estrogen dominance may be observed when a person takes estrogen replacement therapy (ERT) but not (or inadequate) progesterone therapy.

OVERVIEW

The DUTCH Test measures two progesterone metabolites in the urine: a-pregnanediol and b-pregnanediol, see **Figure 3.6**.

Urinary levels of these progesterone metabolites have been shown to strongly correlate with serum progesterone levels when progesterone is not supplemented [j]. a-Pregnanediol modulates GABA receptors in the central nervous system (CNS) and can lead to improvements in mood and sleep. b-Pregnanediol is the major progesterone metabolite, but unlike a-pregnanediol, it does not modulate GABA receptors.

Progesterone's Role in Postmenopausal Females

In postmenopausal females, progesterone supports sleep, mood, and memory, and protects against endometrial cancer, osteoporosis, and cardiovascular disease. When estrogen replacement therapy is used, oral micronized progesterone (OMP) and vaginal micronized progesterone (VMP) protect the endometrium without increasing breast cancer risk during the first 5 years of use.

Progesterone and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Studies evaluating interactions between the provoked HPA axis and progesterone are limited in both healthy and diseased individuals, however it appears that adrenal progesterone increases in response to stress and increased cortisol output. [k]

Progesterone Production in Postmenopausal Females

In postmenopausal females, progesterone is predominantly made in the adrenal glands.

Progesterone to Estrogen Ratio

The progesterone to estrogen ratio can be calculated using b-pregnanediol (the most prominent progesterone metabolite) and E2.

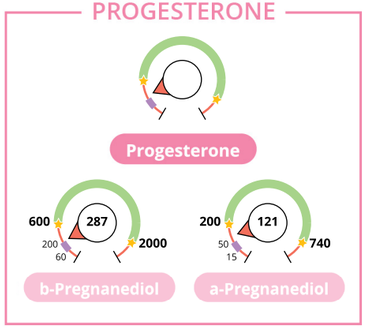
- Pg/E2 ratio = b-Pregnanediol/E2

The ratio is typically 50-300 in the follicular phase, <100 during ovulation and 100-500 in the luteal phase.

NOTE

> *Some providers have found clinically that females may have less symptoms of estrogen dominance if the ratio is closer to 400-500 in the luteal phase.*

Figure 3.6: Measuring Progesterone



The DUTCH Test measures progesterone (Pg) indirectly by taking the weighted average of two metabolites, a-pregnanediol and b-pregnanediol.

[j] Newman M, et al. Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC-MS/MS). BMC Chem. 2019; 13(1): 20.

[k] Herrera AY, et al. Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol Stress. 2016 Feb; 11(3):96-104.

PROGESTERONE IS LOW

Signs & Symptoms

Low Pg in PMP females who are not prescribed MHT may result in fatigue, insomnia, irritability, anxiety, weight gain, and low bone mineral density.

In PMP females with a uterus who are prescribed MHT, Pg dosing must balance E2's endometrial proliferative effects. When Pg dosing is inadequate, PMP females may be at increased endometrial cancer risk.

Potential Root Causes

In PMP females, Pg is predominantly made by the adrenal glands.

In PMP females who are not prescribed MHT, HPA axis dysfunction may contribute to low Pg levels.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other considerations include bioidentical Pg supplementation, and/or HPA axis support with adrenal adaptogens, see **"Appendix B: Potential Support Considerations" on page 149** for more information.

PROGESTERONE IS ELEVATED

Signs & Symptoms

PMP females not on MHT, with Pg metabolites that are above the expected PMP range, but below a cycling female's luteal range, may still experience low Pg symptoms, i.e., fatigue, insomnia, irritability, anxiety, and weight gain.

Females supplementing with Pg may have high Pg symptoms, i.e., fatigue, mood disturbance, bloating, and breast tenderness.

Potential Root Causes

Pg metabolites in PMP females who are not taking MHT that are above the expected PMP range may be secondary to HPA axis dysfunction (stress).

In PMP females prescribed oral/sublingual Pg or pregnenolone, Pg metabolites are typically elevated above the postmenopausal and premenopausal reference ranges[33].

Potential Support Considerations

As Pg is primarily produced in the adrenal glands in PMP females, HPA axis support may be beneficial, see **"Appendix B: Potential Support Considerations" on page 149** for more information.

PROGESTERONE IS WITHIN RANGE

Bioidentical Pg supplementation may still be appropriate for improving energy, sleep, and/or mood even when Pg is within the expected postmenopausal range. OMP or VMP is necessary to balance E2's proliferative effects, especially in females with a uterus, even without E2 supplementation. OMP or VMP are also necessary for improved bone mineral density.

ORAL PROGESTERONE REFERENCE RANGES

Oral micronized progesterone (OMP) age-dependent reference ranges for females:

- a-Pregnanediol: 2,000-9,000 ng/mg
- b-Pregnanediol: 580-3,000 ng/mg

Urinary Pg metabolite levels strongly correlate with serum Pg only when Pg is not being supplemented.

When a female reports taking oral Pg within 72 hours of sample collection, we adjust the Pg reference ranges to reflect the a-pregnanediol and b-pregnanediol levels that are typically seen when a standard dose of 100mg is taken during the test and not skipped.

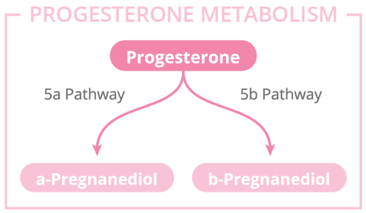
Pg that is within the oral Pg reference range does not guarantee that the dose and ROA of Pg is appropriate and thus does not guarantee endometrial protection when estrogen is also being supplemented.

Serum equivalent values reported on page 1 of the DUTCH Complete and DUTCH Plus reports will be higher than reality when Pg is taken orally or sublingually.

When oral Pg is supplemented, it can be helpful to see if a-pregnanediol is favored, as the alpha Pg metabolites can act on GABA receptors and help with sleep and mood.

PROGESTERONE METABOLISM

Figure 3.7: 5a vs 5b Progesterone Preference



It may be helpful to know if there is a 5a or 5b preference because the alpha progesterone metabolites modulate GABA receptors and may help with mood and sleep.

OVERVIEW

The DUTCH Test measures progesterone indirectly by taking the weighted average of a-pregnanediol and b-pregnanediol, see **Figure 3.7**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

Progesterone is metabolized into a-pregnanediol by 5a-reductase and b-pregnanediol by 5b-reductase. It may be helpful to know if there is a 5a or 5b preference because the alpha progesterone metabolites modulate GABA receptors and may help with mood and sleep. When assessing for an alpha or beta preference, compare the direction of the dials rather than the numbers within the dials.

5a-Reductase and 5b-reductase are also involved in androgen (i.e., DHEA and testosterone) and cortisol metabolism. Therefore, the body's overall preference towards alpha or beta metabolism can be further assessed by comparing alpha vs. Beta metabolite levels. Alpha metabolites on the DUTCH Test include a-pregnanediol, androsterone, 5a-DHT, 5a-androstanediol, and a-THF. Beta metabolites on the DUTCH Test include b-pregnanediol, etiocholanolone, 5b-androstanediol, b-THF, and b-THE.

CLINICAL SIGNIFICANCE

a-Pregnanediol Preference

a-Pregnanediol is a GABA receptor modulator that supports mood and sleep. There is some evidence that a a-pregnanediol preference may increase breast cancer risk, however, more studies are needed to clarify this relationship.

Increased 5a-reductase activity is associated with inflammation, insulin resistance, obesity, DHEA supplementation, and certain genetic makeups. Females supplementing with oral Pg may see improvements in mood and sleep if they favor the alpha pathway.

b-Pregnanediol Preference

b-Pregnanediol is the major Pg metabolite, but unlike a-pregnanediol, it does not exert calming effects in the brain through GABA receptor modulation.

5b-Reductase activity is increased by insulin resistance, high triglycerides, and certain genetic makeups. People may also favor the beta pathway if they are taking a prescription 5a-blocker (finasteride, dutasteride, etc.)[34] or natural 5a-blockers.

When oral Pg is supplemented, females favoring the beta pathway may need a higher Pg dose than females favoring the alpha pathway to see similar improvements in mood and sleep. In these cases, GABA support can also be considered. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

TESTOSTERONE

OVERVIEW

The DUTCH Test reflects bioavailable testosterone. Testosterone's activity may be evaluated by looking at its downstream metabolites, 5a-DHT, 5a-androstanediol, and 5b-androstanediol, see **Figure 3.8**.

In target tissues, testosterone is activated to 5a-DHT by the enzyme 5a-reductase. 5a-DHT is three times more potent than testosterone and is made and metabolized locally in the tissues to 5a-androstanediol. Therefore, urinary 5a-androstanediol may be a better marker of tissue 5a-DHT activity than urinary 5a-DHT. In addition to 5a-DHE and 5a-androstanediol, the downstream alpha metabolite of DHEA, androsterone, can also be used to suggest tissue androgen activity.

NOTE

Some 5a-DHT and 5a-androstanediol can also be formed from DHEA. Therefore, if 5a-DHT and/or 5a-androstanediol are elevated, it may be helpful to compare total DHEA and testosterone to better understand where the excess androgens are originating from (the adrenals or the ovaries). In females, if DHEA-S or total DHEA is high, the source of excess androgens is likely the adrenal glands. Conversely, if these are within range, but testosterone and its metabolites are high, the source may be ovarian.

Testosterone can also be metabolized down the less androgenic beta pathway. The enzyme 5b-reductase is concentrated in the liver (as opposed to target tissues) and converts testosterone into 5b-androstanediol. This testosterone metabolite does not correlate with classic androgen symptoms such as body hair, libido, muscle mass, and acne due to the low androgenic potential of beta metabolites.

NOTE

Certain genetic profiles can cause testosterone to be falsely low in the urine, see **"UGT Deletion" on page 31** *for more information.*

Testosterone's Role in Postmenopausal Females

In postmenopausal females, testosterone supports muscle, strength, stamina, healthy weight, bone, skin, hair, mood, memory, sexual function, cardiovascular health, and estradiol levels.

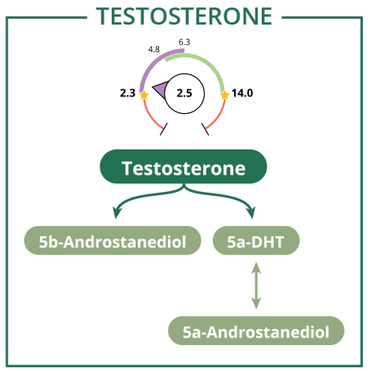
Testosterone Production in Postmenopausal Females

In postmenopausal females, testosterone is mostly derived from peripheral conversion of androstenedione and DHEA. However, a small amount of androstenedione and DHEA are still synthesized in the ovary and can be peripherally converted to testosterone. Testosterone production peaks in the third and fourth decades of life (20s and 30s) and declines thereafter.

Testosterone age-dependent reference ranges for females:

- 20-39 years old: 3.2-14 ng/mg
- 40-60 years old: 2.3-8 ng/mg
- > 60 years old: 1.5-6.3 ng/mg

Figure 3.8: Measuring Testosterone



The DUTCH Test reflects bioavailable testosterone, as well as it's downstream metabolites: 5a-DHT, 5a-androstanediol, and 5b-androstanediol.

TESTOSTERONE IS LOW

NOTE

Individuals with a UGT deletion (genetic variant) may have falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** *for more information on this genetic deletion and the impact on DUTCH testing.*

Signs & Symptoms

Low testosterone in postmenopausal females may be associated with fatigue, weight gain, difficulty building muscle mass, bone loss, mood issues, brain fog, low libido, and sexual dysfunction.

Potential Root Causes

Low testosterone in postmenopausal females may be associated with aging, elevated SHBG, low adrenal output HPA axis dysfunction, poor ovarian production, surgically removed ovaries, diabetes, hypothyroidism, and sleep disturbances, endocrine disrupting chemicals (EDCs), zinc deficiencies, certain medications[35], and upregulated aromatase activity[36].

Low testosterone in females is less commonly associated with traumatic brain injuries, prolactinoma, extremely low body fat percentage, extremely low calorie intake, stress, overexercising, low cholesterol (LDL < 40mg/dL), and suboptimal mitochondrial function.

Potential Support Considerations

In addition to treating the underlying cause, obtain serum total, free and bioavailable testosterone levels and if low consider zinc, herbal testosterone support, HPA axis support, blood sugar regulation, improving sleep, DHEA[37] and/or testosterone replacement therapy[38]. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

If E1 and E2 are elevated, consider natural aromatase inhibition[39].

TESTOSTERONE IS ELEVATED

Signs & Symptoms

Elevated testosterone postmenopausal in females has been associated with acne, oily skin, increased body/facial hair, thinning scalp hair, androgenic alopecia, and mood disturbance (aggression, irritability, etc.).

Mildly elevated androgens that are not accompanied with symptoms of androgen excess may be normal for some women.

Potential Root Causes

Elevated testosterone in postmenopausal females may be associated with low SHBG, downregulated aromatase activity[40], stress, obesity, blood sugar dysregulation, inflammation, testosterone and/or DHEA supplementation[41], transference (from a partner, the gym, etc.), medications[42], or sample contamination[43].

Less common associations include thyroid issues, NCAH, virilizing tumors, and elevated growth hormone.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing inflammation, regulating blood sugar, weight loss if appropriate, supporting the HPA axis, supporting liver detoxification, flaxseeds, myo-inositol, herbal anti-androgens, and medications such as metformin and spironolactone, if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

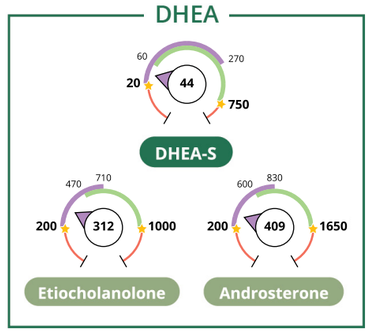
TESTOSTERONE IS WITHIN RANGE

When testosterone is within range, take the alpha androgen levels 5a-DHT, 5a-androstanediol, and androsterone into consideration, as these also have androgenic actions in the body. If there are androgen excess symptoms, but all these androgens are within range or low, consider other causes for these symptoms, such as hypothyroidism, stress, autoimmune issues, mineral deficiencies, etc.

Always confirm DUTCH results with serum testosterone before treatment, as the UGT genetic variant can result in falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** for more information on this genetic deletion and the impact on DUTCH testing.

DHEA

Figure 3.9: Measuring DHEA



The DUTCH Test measures three DHEA metabolites including: DHEA-S, etiocholanolone, and androsterone.

OVERVIEW

The DUTCH Test measures three DHEA metabolites: DHEA-S, etiocholanolone, and androsterone, see **Figure 3.9**.

DHEA is considered a pro-hormone, as most is converted, at the tissue level, to more potent androgens and estrogens. DHEA, in the adrenal gland, is sulfated to DHEA-S, a form that is more stable in serum and can act as a DHEA reserve. The best way to assess the total adrenal androgen production is to add up the DHEA-S, etiocholanolone, and androsterone metabolites, which equals the total DHEA[44] production, also known as the total adrenal androgen production.

NOTE

Total DHEA = DHEA-S + etiocholanolone + androsterone

DHEA's Role in Postmenopausal Females

DHEA supports muscle, bone, sexual function, fertility, brain health, immune function, and cardiovascular health.

DHEA Production in Postmenopausal Females

In postmenopausal females, DHEA is primarily made in the adrenal zona reticularis and almost all estrogens and androgens are derived peripherally from this DHEA [l]. DHEA production peaks in young adulthood (20s) and declines with age.

Total DHEA age-dependent reference ranges for females:

- 20-39 years old: 1300-3000ng/mg
- 40-60 years old: 750-2000ng/mg
- > 60 years old: 500-1200ng/mg

DHEA IS LOW

Signs & Symptoms

Low DHEA in postmenopausal females may be associated with fatigue, low libido, depression, diabetes, heart disease, inflammation, and immune disorders.

Potential Root Causes

Low DHEA in postmenopausal females may be associated with older age, decreased adrenal synthesis (HPA axis dysfunction) secondary to chronic stress, inflammation and inflammatory syndromes, blood sugar dysregulation, hypothyroidism, and medications such as glucocorticosteroids, opioids, budesonide inhalers, and metformin.

Higher DHEA-S relative to lower total DHEA may be associated with upregulated SULT2A1 activity (which sulfates DHEA) or a lower STS enzyme activity (removes the "S" from DHEA-S).

[l] Samaras N, et al. A review of age-related dehydroepiandrosterone decline and its association with well-known geriatric syndromes: is treatment beneficial? Rejuvenation Res. 2013; 16(4): 285-294.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include DHEA replacement therapy[45], addressing chronic inflammation, regulating blood sugar supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, parasympathetic nervous system (PNS) support, and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

DHEA IS ELEVATED

Signs & Symptoms

Elevated DHEA in postmenopausal females has been associated with androgen excess symptoms including oily skin, acne, sleep problems, headaches, and mood disturbances.

Potential Root Causes

Elevated DHEA in postmenopausal females may be associated with DHEA supplementation[46], stress, high adrenal cortisol output, HPA axis dysfunction, inflammation, blood sugar dysregulation, alcohol, nicotine, testosterone supplementation[47], and medications such as Alprazolam, Anastrozole, Methylphenidate, Amlodipine, Diltiazem, and Bupropion.

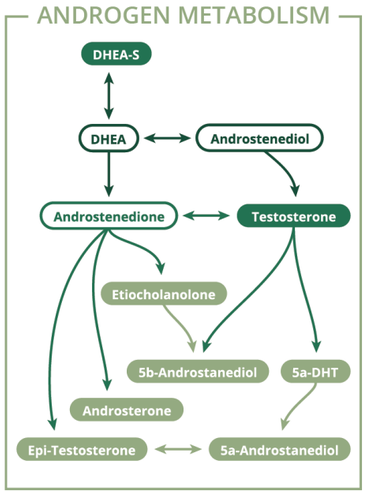
Less common associations include nonclassical congenital adrenal hyperplasia (NCAH), hyperprolactinemia, and an adrenal tumor.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing chronic inflammation, regulating blood sugar, supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, PNS support, and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

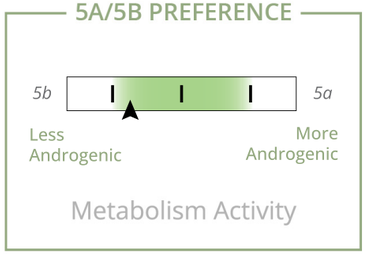
ANDROGEN METABOLISM

Figure 3.10: Androgen Metabolism



The DUTCH Test measures several androgenic metabolites that stem from testosterone and DHEA, to provide a more complete picture of a patients' metabolic health.

Figure 3.11: 5a vs 5b Androgen Preference



It may be helpful to know if there is a 5a or 5b androgen preference because a beta preference may exacerbate signs and symptoms of low androgens (e.g., low libido, fatigue, weight gain), while an alpha preference may exacerbate signs and symptoms of high androgens (e.g., scalp hair loss, acne, body hair growth).

OVERVIEW OF 5-ALPHA VS 5-BETA

The DUTCH Test measures bioavailable testosterone, DHEA-S and their metabolites, see **Figure 3.10**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

Regarding androgen metabolism, a person may favor the more androgenic alpha pathway, the less androgenic beta pathway, or a balance between the two pathways.

5a-Reductase converts DHEA and testosterone into the alpha androgen metabolites: androsterone, 5a-DHT, and 5a-androstanediol. 5a-DHT is testosterone's active metabolite. It is three times more potent than testosterone, and if elevated may contribute to androgen excess symptoms. 5a-DHT is a peripheral hormone, and research shows that 5a-androstanediol may be a better marker of 5a-DHT tissue activity. [m]

5b-Reductase converts DHEA and testosterone into the beta androgen metabolites: etiocholanolone and 5b-androstanediol. The beta androgen metabolites are generally less androgenic than the alpha androgen metabolites and are formed in the liver, not the tissues. Therefore, these beta metabolites are less likely to contribute to androgen excess symptoms. For example, a person who has elevated testosterone may not experience androgen excess symptoms if they metabolize most of their testosterone down the less androgenic beta pathway.

NOTE

The slider on the DUTCH Test, see **Figure 3.11**, *shows the relative ratio of 5a to 5b metabolites but does not express the absolute value of 5a-DHT and 5a-androstanediol, or if 5a-reductase inhibition is or is not indicated. Consider symptoms and look at the 5a-DHT, androsterone and 5a-androstanediol results if high androgen symptoms are a concern.*

5a-Reductase and 5b-reductase are also involved in androgen (i.e., DHEA and testosterone) and cortisol metabolism. Therefore, the body's overall preference towards alpha or beta metabolism can be further assessed by comparing alpha vs. beta metabolite levels. Alpha metabolites on the DUTCH Test include a-pregnanediol, androsterone, 5a-DHT, 5a-androstanediol, and a-THF. Beta metabolites on the DUTCH Test include b-pregnanediol, etiocholanolone, 5b-androstanediol, b-THF, and b-THE.

[m] Trabert B, et al. Progesterone and Breast Cancer. Endocr Rev. 2020 Apr 1; 41(2):320–44.

CLINICAL SIGNIFICANCE

5a-Reductase Preference

A metabolic preference for the alpha pathway may lead to elevated androsterone, 5a-DHT, 5a-androstanediol, and excess androgen symptoms. Inflammation, high stress lifestyles, obesity, and blood sugar dysregulation can upregulate 5a-reductase.

Consider addressing these root causes if the goal is to decrease the alpha preference. Also consider 5a-reductase herbal blockers and medications such as metformin, spironolactone, or finasteride[48], if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

If DHEA and testosterone are low, a metabolic preference for the 5a-reductase enzyme may not necessarily lead to elevated 5a-DHT levels and symptoms of androgen excess.

5b-Reductase Preference

A metabolic preference for the beta pathway may lead to low androsterone, 5a-DHT, 5a-androstanediol, and low androgen symptoms.

Certain genetic makeups, 5a-reductase herbal blockers, and medications such as spironolactone and finasteride[49] may contribute to a beta preference, so consider addressing these root causes if the goal is to decrease the beta preference. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Consider upregulating 5a-reductase activity with high intensity interval training (HIIT), weight resistance exercises, and supplements such as forskolin and pine pollen. Low androgen symptoms associated with low DHEA and/or testosterone may improve by directly addressing DHEA and testosterone levels.

NOTE

If DHEA and testosterone are elevated, a metabolic preference for the beta pathway may be beneficial, as it may help to keep 5a-DHT within range.

ANNOTATIONS

27 Aromatase activity can be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

28 DIM and indole-3-carbinol (I3C) are sometimes used to induce CYP-1a1 (the “preferred” pathway towards 2-OH), however be mindful that they also tend to lower E1 and E2 (Newman, M., Smeaton, J. Exploring the impact of 3,3′-diindolylmethane on the urinary estrogen profile of premenopausal women. BMC Complement Med Ther 24, 405 (2024).) which may not be appropriate for every person. When using DIM and/or I3C, it is important that phase 2 detoxification is well supported so that the phase 1 metabolites do not build up in the body and cause oxidative damage. If on ERT, sometimes a relatively higher dose of estrogen may be needed when used in combination with estrogenlowering compounds such as DIM and/or I3C in order to achieve desired clinical outcomes. If on Tamoxifen, note that DIM and I3C may render Tamoxifen less effective.

29 ERT must be balanced with Pg in females with a uterus. In females who have had a hysterectomy (no uterus), progesterone therapy is optional and may provide support for bone mineral density, mood, and sleep.

30 Aromatase activity can be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

31 Use caution when interpreting E2 and estrogen metabolite results if oral or sublingual E2, testosterone, and/or DHEA was prescribed, as these undergo first-pass metabolism, which results in elevated urinary metabolites that do not correlate with serum levels. We suggest stopping oral DHEA 48 hours prior to collection and oral testosterone and E2 or Biest (E2 and E3)72 hours prior to collection.

32 Estradiol replacement therapy (ERT) must be balanced with progesterone in females with a uterus. In females without a uterus, estradiol and progesterone should also be balanced for optimal bone mineral density and to avoid estrogen dominant symptoms.

33 Urinary progesterone metabolite levels strongly correlate with serum progesterone only when progesterone is not being supplemented. Use caution when interpreting progesterone results if progesterone or pregnenolone was supplemented orally or sublingually within 72 hours of sample collection, as the portion that is swallowed undergoes first-pass metabolism and results in elevated urinary metabolites that do not correlate with serum levels.

34 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

35 Medications that may contribute to low testosterone include glucocorticosteroids, opioids, Accutane, spironolactone, and some oral contraceptive pills (OCPs).

36 Aromatase activity may be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

37 Use caution with oral DHEA supplementation as it may elevate estrogen.

38 Testosterone should always be evaluated and confirmed in the serum before initiating TRT for low testosterone.

39 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

40 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, PCOS, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

41 Use caution when interpreting testosterone results if oral or sublingual DHEA or testosterone were supplemented, as these undergo first-pass metabolism, which results in elevated urinary metabolites that do not correlate with serum levels.

42 Medications that may contribute to elevated testosterone include aromatase inhibitors, clomiphene citrate (a selective estrogen receptor modulator (SERM)), and luteinizing hormone (LH), growth hormone (GH), and gonadotropin-releasing hormone (GnRH) analogs (hCG, gonadorelin, kisspeptin, etc.).

43 If testosterone is elevated but the downstream androgen metabolites are not (5a-DHT, androsterone, 5a-androstanediol, etiocholanolone, and 5b-androstanediol) consider the possibility of testosterone sample contamination from someone using TRT (testosterone replacement therapy).

44 Total DHEA is found on the cover and page 5 of a DUTCH Complete and DUTCH Plus report. Note that the Adrenal Only panel measures DHEA-S but not etiocholanolone and androsterone. Total DHEA is calculated by adding together DHEA-S + etiocholanolone + androsterone. For a sex hormone panel, total DHEA is not already calculated; therefore, calculate the total DHEA and use the age-dependent reference ranges above.

45 Use caution with oral DHEA supplementation, as it may elevate estrogens in addition to increasing testosterone.

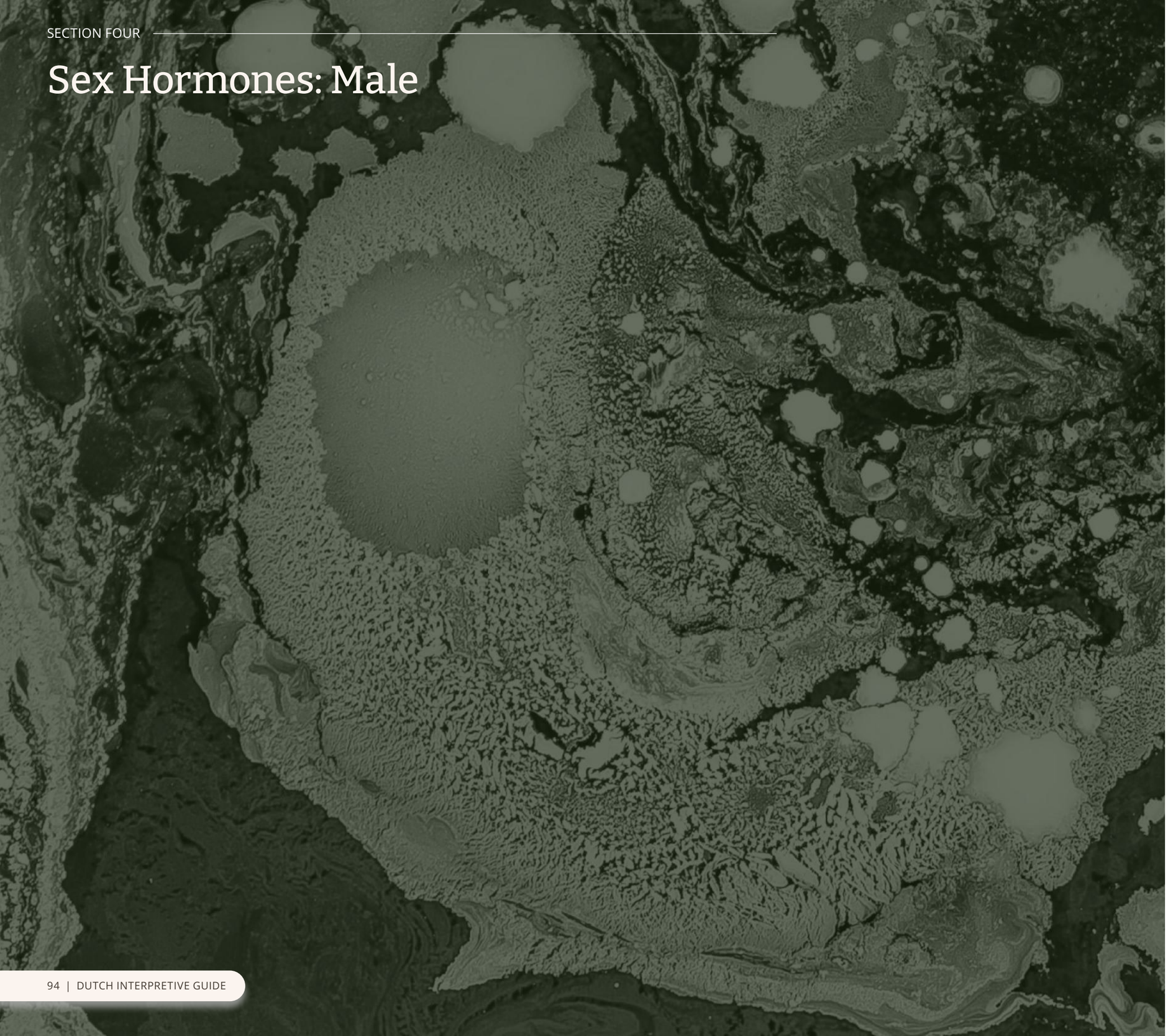
46 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into, and elevate, androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Oral DHEA can create false elevations when used during testing.

47 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into, and elevate, androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Oral DHEA can create false elevations when used during testing.

48 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

49 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

Sex Hormones: Male



Introduction

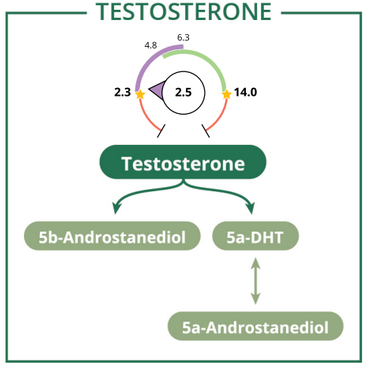
The DUTCH Test has a breadth of clinical utility in males. While testosterone measurements are best done in serum to obtain quantitative measures, the DUTCH Test offers additional insight into a male's overall reproductive hormonal picture, as well as androgen metabolites, which often can induce symptoms of excess or be responsible for symptoms of deficiency.

You may be surprised that other hormones affect males as well, including estrogen and estrogen metabolites. In this guide, you will learn about interpretation of the DUTCH Test in males, including how to make sense of elevated or low levels of hormones and metabolites.

LET'S GET STARTED ▶▶

TESTOSTERONE

Figure 4.1: Measuring Testosterone



The DUTCH Test reflects bioavailable testosterone, as well as it's downstream metabolites: 5a-DHT, 5a-androstanediol, and 5b-androstanediol.

OVERVIEW

The DUTCH Test reflects bioavailable testosterone. Testosterone's activity may be evaluated by looking at its downstream metabolites, 5a-DHT, 5a-androstanediol, and 5b-androstanediol, see **Figure 4.1**.

In target tissues, testosterone is activated to 5a-DHT by the enzyme 5a-reductase. 5a-DHT is three times more potent than testosterone and is made and metabolized locally in the tissues to 5a-androstanediol. Therefore, urinary 5a-androstanediol may be a better marker of tissue 5a-DHT activity than urinary 5a-DHT. In addition to the 5a-DHT and 5a-androstanediol, the downstream alpha metabolite of DHEA (androsterone) can additionally be used to evaluate tissue androgen activity.

NOTE

Some 5a-DHT and 5a-androstanediol can also be formed from DHEA. Therefore, 5a-DHT and/or 5a-androstanediol are elevated, it may be helpful to compare total DHEA and testosterone to better understand where the excess androgens are originating from (the adrenals or the testes). In males, testosterone is made almost exclusively in the testes, whereas DHEA is made primarily in the adrenal glands. Thus, if DHEA is elevated in a male, the source of excess androgens is likely the adrenals, but if testosterone or its metabolites are elevated, the source is likely the testes.

Testosterone can also be metabolized down the less androgenic beta pathway. The enzyme 5b-reductase is concentrated in the liver (as opposed to target tissues) and converts testosterone into 5b-androstanediol. This testosterone metabolite does not correlate with classic androgen symptoms such as body hair, libido, muscle mass, and acne due to the low androgenic potential of beta metabolites.

NOTE

Certain genetic profiles can cause testosterone to be falsely low in the urine, see **"UGT Deletion" on page 31** *for more information.*

Testosterone's Role in Males

In males, testosterone supports muscle, strength, stamina, healthy weight, bone, skin, hair, mood, memory, sexual function, sperm production, fertility, cardiovascular health, and estradiol levels.

Testosterone Production in Males

In males, 95% of testosterone is made by the testes. Testosterone peaks at approximately 20 years of age and starts to decline during the fourth decade of life (30s). Testosterone levels in healthy males vary, so it is critical to consider the person's symptoms as a point of context when interpreting DUTCH Test results.

Testosterone age-dependent reference ranges for males:

- 18-25 years old: 35-115 ng/mg
- 26-40 years old: 30-95 ng/mg
- 41-60 years old: 25-80 ng/mg
- > 60 years old: 20-60 ng/mg

TESTOSTERONE IS LOW

NOTE

Individuals with a UGT deletion (genetic variant) may have falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** *for more information on this genetic deletion and the impact on DUTCH testing.*

Signs & Symptoms

Low testosterone in males may been associated with fatigue, weight gain, decreased muscle mass, difficulty building muscle, erectile dysfunction, low libido, bone loss, mood issues, brain fog, and gynecomastia (if estradiol is elevated).

Potential Root Causes

Low testosterone in males may be associated with diabetes, obesity, HPA axis dysfunction (high cortisol), hypothyroidism, older age, poor sleep, endocrine disrupting chemicals (EDCs), zinc deficiency, vitamin D deficiency, high alcohol use, regular THC use, recent testosterone supplementation, elevated SHBG, increased aromatization[50], and certain medications[51].

Less common associations include traumatic brain injuries, hyperprolactinemia, pituitary and hypothalamic disease, testicular infection, autoimmune antibodies (Leydig cell specific), groin radiation, chemo at-large, and leptin and leptin receptor mutations.

Potential Support Considerations

In addition to treating the underlying cause, obtain a serum total, free, and bioavailable testosterone. If serum testosterone is low other considerations include high dose DHEA[52] or testosterone replacement therapy (TRT) in males > 50 years old. In young males or older males wanting to maintain fertility consider clomiphene, gonadorelin, and kisspeptin. HCG may be considered for males with documented fertility issues.

Also consider zinc, herbal testosterone support, HPA axis support, high intensity interval training (HIIT) and weightlifting to increase muscle mass, blood sugar regulation, and improving sleep. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

If E1 and E2 are elevated, consider aromatase inhibition with herbal and/or pharmaceutical agents.

TESTOSTERONE IS ELEVATED

Signs & Symptoms

In males, elevated endogenous testosterone has been associated with acne, oily skin, increased body, and facial hair, thinning scalp hair, androgenic alopecia, and mood disturbance (aggression, irritability, etc.)

When high testosterone is due to TRT, it may cause low sperm counts (negative feedback), mood swings, aggression, increased hematocrit, and increased E2. Increased HCT (> 50%) and increased E2 levels are associated with increased clotting risk. If elevated testosterone increases DHT, this may increase the likelihood of BPH symptoms.

NOTE

TRT neither increases cardiovascular disease, nor prostate cancer risk.

Potential Root Causes

Elevated testosterone in males may be normal, especially in young males, or more commonly due to low SHBG, testosterone and DHEA (high dose) supplementation[53], sample contamination[54], and certain medications[55], and downregulated aromatase activity[56].

Less common associations include elevated growth hormone (GH), nonclassical congenital adrenal hyperplasia (NCCAH), and virilizing tumors.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing inflammation, regulating blood sugar, weight loss if appropriate, supporting the HPA axis, supporting liver detoxification, flaxseeds, herbal anti-androgens, and medications such as metformin, spironolactone and/or finasteride, if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

In males, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

TESTOSTERONE IS WITHIN RANGE

When testosterone is within range, take into consideration the other DUTCH Test androgen levels (DHEA, 5a-DHT, 5a-androstanediol, 5b-androstanediol, etiocholanolone, and androsterone), as these also have androgenic action in the body. If there are androgen excess or deficiency symptoms but androgens are within range on the DUTCH Test, consider other causes, e.g., hypothyroidism, stress, autoimmune issues, mineral deficiencies, etc. In males, elevated estradiol can mimic low androgen symptoms.

Always confirm serum testosterone results (total testosterone, free testosterone (equilibrium dialysis or calculated), and bioavailable testosterone) before treatment, as the genetic variant UGT deletion can result in falsely low urinary testosterone, 5a-DHT, and 5b-androstanediol. Please see **"UGT Deletion" on page 31** for more information on this genetic deletion and the impact on DUTCH testing.

OVERVIEW

The DUTCH Test measures three DHEA metabolites: DHEA-S, etiocholanolone, and androsterone, see **Figure 4.2**.

DHEA is considered a pro-hormone, as most is converted, at the tissue level, to more potent androgens and estrogens. DHEA, in the adrenal gland, is sulfated to DHEA-S, a form that is more stable in serum and can act as a DHEA reserve. The best way to assess the total adrenal androgen production is to add up the DHEA-S, etiocholanolone, and androsterone metabolites, which equals the total DHEA production, also known as the total adrenal androgen production.

NOTE

Total DHEA = DHEA-S + etiocholanolone + androsterone

DHEA's Role in Males

DHEA supports muscle, bone, sexual function, fertility, brain health, immune function, and cardiovascular health.

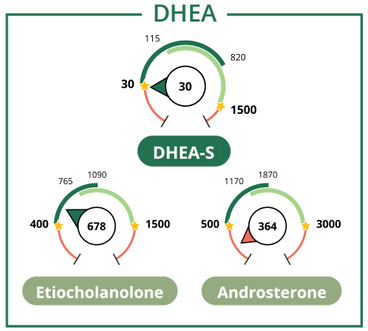
DHEA Production in Males

In males, most DHEA is produced by the adrenal glands, however about 20% is produced by the testes. The adrenal glands produce 100% of DHEA-S. DHEA production peaks in young adulthood (20s) and declines with age.

Total DHEA age-dependent reference ranges for males:

- 20-39 years old: 3000-5500ng/mg
- 40-60 years old: 2000-4000ng/mg
- > 60 years old: 1000-2500ng/mg

Figure 4.2: Measuring DHEA



The DUTCH Test measures three DHEA metabolites including: DHEA-S, etiocholanolone, and androsterone.

DHEA IS LOW

Signs & Symptoms

Low DHEA in males may be associated with fatigue, low libido, depression, diabetes, heart disease, inflammation, and immune disorders.

Potential Root Causes

Low DHEA in males may be associated with older age, low adrenal output, HPA axis dysfunction secondary to chronic stress, inflammation, blood sugar dysregulation, hypothyroidism, and medications such as glucocorticosteroids, opioids, budesonide inhalers, and metformin.

Higher DHEA-S relative to lower total DHEA may be due to upregulated SULT2A1 activity (which sulfates DHEA) or lower STS enzyme activity (removes the "S" from DHEA-S).

Potential Support Considerations

In addition to treating the underlying cause, other considerations include DHEA replacement therapy, addressing chronic inflammation, regulating blood sugar,

supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, parasympathetic nervous system (PNS) support, and GABA support. See **“Appendix B: Potential Support Considerations” on page 149** for more information.

NOTE

Use caution with oral DHEA supplementation, as it may elevate estrogens in males.

DHEA IS ELEVATED

Signs & Symptoms

Elevated DHEA in males may be associated with androgen excess symptoms including oily skin, acne, sleep problems, headaches, and mood disturbances.

Potential Root Causes

Elevated DHEA in a males may be associated with DHEA supplementation[57], stress, high adrenal cortisol output, HPA axis dysfunction, inflammation, blood sugar dysregulation, alcohol, nicotine, testosterone supplementation[58], and medications such as Alprazolam, Anastrozole, Methylphenidate, Amlodipine, Diltiazem and Bupropion.

Less common associations include NCAH, hyperprolactinemia, and an adrenal tumor.

Potential Support Considerations

In addition to treating the underlying cause, other considerations include addressing chronic inflammation, regulating blood sugar, supporting liver detoxification, HPA axis support, and stress reduction, which may include calming herbal support, PNS support, and GABA support. See **“Appendix B: Potential Support Considerations” on page 149** for more information.

OVERVIEW OF 5-ALPHA VS 5-BETA

The DUTCH Test measures bioavailable testosterone, DHEA-S and their metabolites, see **Figure 4.3**. For a more complete view of metabolism, see **“The DUTCH Steroid Pathway” on page 47**.

Regarding androgen metabolism, a person may favor the more androgenic alpha pathway, the less androgenic beta pathway, or be balanced between the two pathways, see **Figure 4.4**.

5a-Reductase converts DHEA and testosterone into the alpha androgen metabolites: androsterone, 5a-DHT, and 5a-androstanediol. 5a-DHT is testosterone’s active metabolite. It is three times more potent than testosterone, and if elevated may contribute to androgen excess symptoms. 5a-DHT is a peripheral hormone, and research shows that 5a-androstanediol may be a better marker of 5a-DHT tissue activity. [n]

5b-Reductase converts DHEA and testosterone into the beta androgen metabolites: etiocholanolone and 5b-androstanediol. The beta androgen metabolites are generally less androgenic than the alpha androgen metabolites. Therefore, these beta metabolites are less likely to contribute to androgen excess symptoms. For example, a person who has elevated testosterone may not experience androgen excess symptoms if they metabolize most of their testosterone down the less androgenic beta pathway.

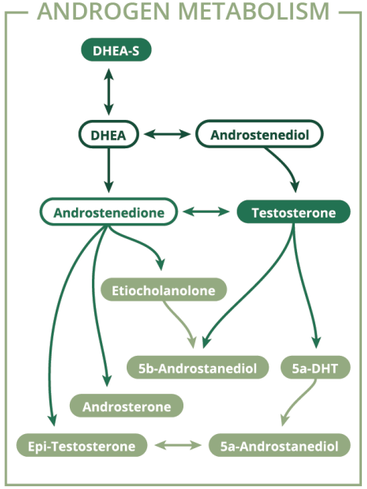
NOTE

The slider on the DUTCH Test, see **Figure 4.4***, shows the relative ratio of 5a to 5b metabolites but does not express the absolute value of 5a-DHT and 5a-androstanediol, or if 5a-reductase inhibition is or is not indicated. Consider symptoms and look at the 5a-DHT, androsterone and 5a-androstanediol results if high androgen symptoms are a concern.*

5a-Reductase and 5b-reductase are also involved in androgen (i.e., DHEA and testosterone) and cortisol metabolism. Therefore, the body’s overall preference towards alpha or beta metabolism can be further assessed by comparing alpha vs. beta metabolite levels. Alpha metabolites on the DUTCH Test include a-pregnanediol, androsterone, 5a-DHT, 5a-androstanediol, and a-THF. Beta metabolites on the DUTCH Test include b-pregnanediol, etiocholanolone, 5b-androstanediol, b-THF, and b-THE.

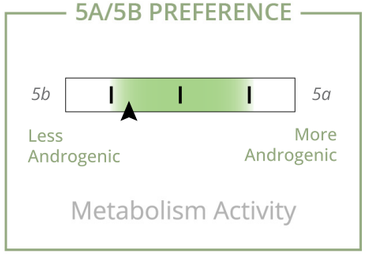
[n] Trabert B, et al. Progesterone and Breast Cancer. Endocr Rev. 2020 Apr 1; 41(2):320–44.

Figure 4.3: Androgen Metabolism



The DUTCH Test measures several androgenic metabolites that stem from testosterone and DHEA, to provide a more complete picture of a patients’ metabolic health.

Figure 4.4: 5a vs 5b Androgen Preference



It may be helpful to know if there is a 5a or 5b androgen preference because a beta preference may exacerbate signs and symptoms of low androgens such as low libido, fatigue, and weight gain.

CLINICAL SIGNIFICANCE

5a-Reductase Preference

A metabolic preference for the alpha pathway may lead to elevated androsterone, 5a-DHT, 5a-androstanediol, and excess androgen symptoms. Inflammation, high stress lifestyle, obesity, and blood sugar dysregulation can upregulate 5a-reductase.

Consider addressing these root causes if the goal is to decrease the alpha preference. Also consider 5a-reductase herbal blockers and medications such as metformin, spironolactone, or finasteride[59], if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

> *If DHEA and testosterone are low, a metabolic preference for the 5a-reductase enzyme may not necessarily lead to elevated 5a-DHT levels and symptoms of androgen excess.*

In males, elevated 5a-DHT may lead to BPH, while elevated estradiol, especially in the setting of inflammation, may impact prostate cancer development.

5b-Reductase Preference

A metabolic preference for the beta pathway may lead to low androsterone, 5a-DHT, 5a-androstanediol, and low androgen symptoms.

Certain genetic makeups, 5a-reductase herbal blockers, and medications such as spironolactone and finasteride[60] may contribute to a beta preference, so consider addressing these root causes if the goal is to decrease the beta preference. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Consider upregulating 5a-reductase activity with HIIT, weight resistance exercises, and supplements such as forskolin and pine pollen. Low androgen symptoms associated with low DHEA and/or testosterone may improve by directly addressing DHEA and testosterone levels.

NOTE

> *If DHEA and testosterone are elevated, a metabolic preference for the beta pathway may be beneficial, as it may help to keep 5a-DHT within range.*

OVERVIEW

The DUTCH Test measures the three primary estrogens: Estrone (E1), Estradiol (E2), and Estriol (E3), see **Figure 4.5**.

Estradiol has the strongest stimulatory effect on estrogen receptors while estriol has the weakest effect. Estrone and estradiol can interconvert via 17b-hydroxy steroid dehydrogenase (HSD) and estriol is an estradiol and 16-OH-E1 downstream metabolite.

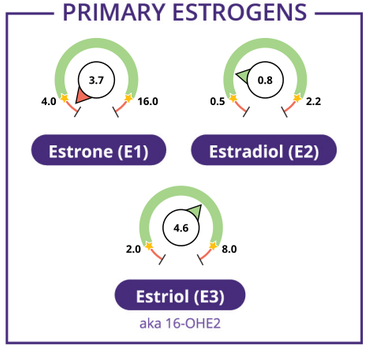
Estrogen's Role in Males

Over the past few decades research has clarified the importance of healthy estrogen levels and a balanced estrogen to testosterone ratio in males. Estradiol that is well balanced with testosterone, is beneficial for bone health, healthy weight-management, mood, concentration, memory, hair, skin, cardiovascular health, libido, and the ability to obtain and maintain an erection.

Estrogen Production in Males

The testes produce approximately 20% of E2 and the remaining 80% is aromatized from androgens in adipose (fat) tissue, muscle, breast, brain, liver, and bone. Therefore, most of the estrogen in males is aromatized from testosterone, androstenedione, and DHEA in the periphery.

Figure 4.5: Measuring Estrogen



The DUTCH Test measures the three primary estrogens: Estrone, Estradiol, and Estriol.

ESTRADIOL IS LOW

Signs & Symptoms

Low E2 in males has been associated with, an unfavorable lipid profile, decreased bone mineral density (even when total testosterone levels are within the normal range), erectile dysfunction, and infertility.

Low E2 in males may also result in low testosterone symptoms because E2 potentiates testosterone receptors.

Potential Root Causes

Low E2 in males is primarily due to low testosterone production, HPA axis dysfunction (high cortisol), and downregulated aromatase activity[61], and diindolylmethane (DIM)/I3C[62].

NOTE

> *Males with a genetic aromatase polymorphism may present, at a young age, with bone fractures and a bone mineral density reveals osteopenia/osteoporosis.*

In males with either congenital or acquired hypogonadotropic hypogonadism, E2 will be low-normal or low.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other support considerations include: for males < 50 years old herbal testosterone support, Clomid, peptides, and if fertility is an issue, hCG; TRT for males > 50 years old.

NOTE

Testosterone should always be evaluated and confirmed in the serum before initiating testosterone replacement therapy (TRT).

Additional considerations may also include supporting the HPA axis, and blood sugar regulation, see **"Appendix B: Potential Support Considerations" on page 149** for more information.

ESTRADIOL IS ELEVATED

Signs & Symptoms

Elevated E2 levels in males has been associated with breast tissue enlargement, obesity, mood changes, low sex drive, and impaired erectile function.

Potential Root Causes

In males, elevated E2 levels are commonly associated with TRT at supraphysiologic doses, obesity, glucose and insulin dysregulation, inflammation, poor detoxification, endocrine disrupting chemicals (EDCs), upregulated aromatase activity[63], and high alcohol intake.

Interpret DUTCH results with caution if oral or sublingual hormone replacement therapy (HRT) is being supplemented[64].

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other support considerations include inhibiting aromatase naturally or with pharmaceutical aromatase inhibitors such as anastrozole, supporting estrogen detoxification and metabolism patterns, supporting the HPA axis, lowering inflammation, regulating blood sugar, weight loss (if appropriate), lowering androgens if elevated, and general liver support to further encourage hormone detoxification. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

OVERVIEW

The DUTCH Test measures two progesterone metabolites in the urine: a-pregnanediol and b-pregnanediol, see **Figure 4.6**.

Urinary levels of progesterone metabolites have been shown to strongly correlate with serum progesterone levels when progesterone is not supplemented. [o] a-pregnanediol is a central nervous system (CNS) GABA receptor modulator and can lead to improvements in mood and sleep. b-Pregnanediol is the major progesterone metabolite, but unlike a-pregnanediol, it does not modulate GABA receptors.

Progesterone's Role in Males

In males, progesterone's role is not well understood. Progesterone influences sperm production and activation as well as testicular Leydig cell testosterone biosynthesis. It has neuroprotective properties and may improve sleep and mood through alpha progesterone metabolite modulation of GABA receptors. Elevated progesterone is associated with inflammation and stress.

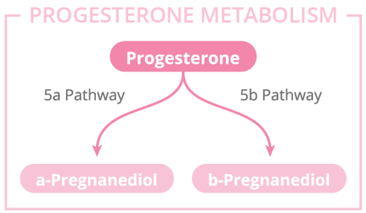
Progesterone and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Studies evaluating interactions between the provoked HPA axis and progesterone are limited in both healthy and diseased individuals, however adrenal progesterone increases in response to stress and increased cortisol output. [p]

Progesterone Production in Males

In males, progesterone is primarily made in the adrenal glands, however testicular Leydig cells also synthesize some progesterone.

Figure 4.6: Measuring Progesterone



The DUTCH Test measures two progesterone metabolites in the urine, a-pregnanediol and b-pregnanediol.

PROGESTERONE IS LOW

Signs & Symptoms

In males, low Pg may result in infertility.

Potential Root Causes

In males the effect of low Pg is not well understood. It is plausible that low Pg may signal low overall adrenal production and/or low testicular Pg production.

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other considerations include herbal testicular support with tongkat ali, ashwagandha, mucuna, Tribulus, fenugreek, and HPA axis support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

[o] Newman M, et al. Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC-MS/MS). BMC Chem. 2019; 13(1): 20.

[p] Herrera AY, et al. Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol Stress. 2016 Feb 11; 3:96-104.

PROGESTERONE IS ELEVATED

Signs & Symptoms

There is little research on the effect of high Pg in males.

Potential Root Causes

In males, elevated Pg may be associated with elevated cortisol, increased inflammation, and liver disease. High Pg may also be due to pregnenolone/progesterone supplementation[65].

Potential Support Considerations

In addition to assessing and treating the underlying root cause, other considerations include stress reduction and HPA axis support. See **“Appendix B: Potential Support Considerations” on page 149** for more information.

ANNOTATIONS

50 Aromatase activity can be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

51 Medications that can contribute to lower testosterone include performance steroids, glucocorticosteroids, opioids, Accutane, and anti-androgen therapies.

52 Use caution with oral DHEA supplementation, as it may elevate estrogens (estradiol and estrone) in males.

53 Use caution when interpreting testosterone results if oral or sublingual DHEA or testosterone was supplemented, as these undergo first-pass metabolism, which results in elevated urinary metabolites that do not correlate with serum levels. Androgen supplementation can suppress endogenous testosterone (and epi-testosterone) production in a dose-dependent manner. In these cases, epi-testosterone may provide a rough approximation of endogenous testosterone production.

54 If testosterone is elevated but the downstream androgen metabolites are not (5a-DHT, 5a-androstanediol, 5b-androstanediol), consider the possibility of sample contamination with testosterone from someone in the house or gym using TRT.

55 Medications that may contribute to elevated testosterone include aromatase inhibitors, luteinizing hormone (LH), growth hormone (GH), gonadotropin-releasing hormone (GnRH) analogs (hCG, gonadorelin, kisspeptin, etc.), and clomiphene citrate (a SERM, selective estrogen receptor modulator).

56 Aromatase activity can be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

57 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into, and elevate, androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Oral DHEA can create false elevations when used during testing.

58 Use caution when interpreting total DHEA if testosterone replacement therapy (TRT) and/or DHEA was taken. Testosterone can metabolize into, and elevate, androsterone and etiocholanolone. As total DHEA is calculated by adding up DHEA-S + etiocholanolone + androsterone, TRT (or even high endogenous testosterone) may elevate this calculated marker. Additionally, oral, or sublingual supplemented testosterone and DHEA undergo first-pass metabolism in the gut and liver, which results in elevated urine metabolites that do not correlate with serum levels. Oral DHEA can create false elevations when used during testing.

59 In males and females, finasteride and dutasteride may cause irreversible sexual dysfunction and infertility.

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61 Aromatase activity may be downregulated by enterolactone, apigenin, chrysin and other flavonoids, white button mushrooms, grape seed extract, red wine procyanidin dimers, antifungal medications, metformin, glyphosate, smoking, antiepileptic drugs, and pharmaceutical aromatase inhibitors. Genetic SNPs in CYP19A1 may also affect aromatase activity.

62 Diindolylmethane (DIM) and indole-3-carbinol (I3C) are sometimes used in order to induce CYP-1a1 (the “preferred” pathway towards 2-OH), however be mindful that they also tend to lower E1 and E2 (Newman, M., Smeaton, J. Exploring the impact of 3,3’-diindolylmethane on the urinary estrogen profile of premenopausal women. BMC Complement Med Ther 24, 405 (2024).) which may not be appropriate for every patient. When using DIM and/or I3C, it is important that phase 2 detoxification is well supported so that the phase 1 metabolites do not build up in the body and cause oxidative damage.

63 Aromatase activity can be upregulated by obesity, inflammation, high insulin, forskolin, quercetin, genistein (bioflavonoids), white peony and licorice root, atrazine, and rutin. Genetic SNPs in CYP19A1 may also affect aromatase activity.

64 Use caution when interpreting E2 results if oral or sublingual estrogen, DHEA or testosterone was supplemented, as these undergo first-pass metabolism, which results in elevated urinary metabolites that do not correlate with serum levels. DUTCH suggests stopping oral DHEA 48 hours prior to collection and estrogen and testosterone 72 hours prior to collection.

65 Use caution when interpreting progesterone metabolite results with oral or sublingual progesterone or pregnenolone, as these undergo first pass-metabolism, which results in elevated urinary metabolites that do not correlate with serum levels.

SECTION FIVE

Estrogen Detoxification

Introduction

Healthy estradiol metabolism and clearance is important for regulating estradiol levels and reducing female and male cancer risk (endometrial and prostate, respectively).

NOTE

For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

Phase 1 Detox

In phase 1, E1 and E2 are hydroxylated into 2-hydroxy (2-OH), 4-hydroxy (4-OH), and 16-hydroxy (16-OH) metabolites. These intermediate metabolites require further processing to be ready for elimination.

NOTE

If phase 1 metabolites are much lower relative to their parent estrogens (E1 and E2), this implies sluggish phase 1 clearance of estrogens, which can contribute to estrogen excess.

Oppositely, if the phase 1 metabolites are much higher relative to their parent estrogens (E1 and E2), then this implies fast phase 1 clearance of estrogens, which can contribute to estrogen deficiency. Phase 1 detoxification, for example, is upregulated by products containing DIM or I3C. This pattern can also occur with sluggish phase 2 metabolism, however.

Phase 2 Detox

In phase 2, the phase 1 estrogen metabolites are methylated, sulfated, glucuronidated and conjugated with glutathione. For example, 2-hydroxy-estrone is methylated to 2-methoxy-estrone, a compound that is more stable and ready for excretion from the body.

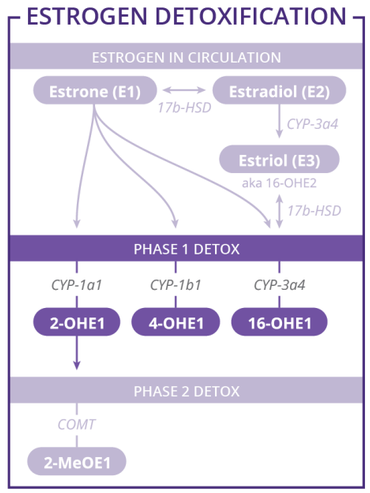
Phase 3 Detox

In phase 3, estrogens are eliminated in bile (stool) and urine.

LET'S GET STARTED ▸▸

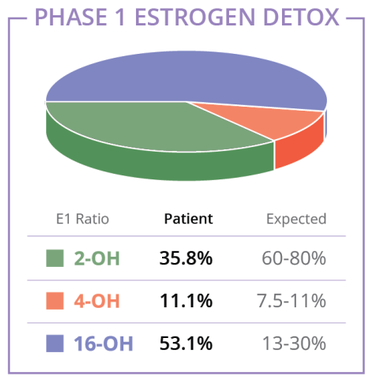
PHASE 1 DETOX

Figure 5.1: Phase 1 Detox



During phase 1, E1 and E2 are hydroxylated into 2-OH, 4-OH, and 16-OH metabolites.

Figure 5.2: Phase 1 Detox Ratios



The DUTCH Test shows a pie chart that visually demonstrates the ratios of the 2-OH, 4-OH, and 16-OH metabolites, to see which pathways are metabolically preferred.

OVERVIEW

During phase 1, E1 and E2 are hydroxylated into 2-OH, 4-OH, and 16-OH metabolites, see **Figure 5.1**. For a more complete view of metabolism, see **"The DUTCH Steroid Pathway" on page 47**.

2-OH catechol estrogens are the most stable of the phase 1 metabolites. The 4-OH pathway is the least stable and can form reactive quinones that bind to DNA, causing damage. This can increase breast cancer risk, over time. The 16-OH catechol estrogens have small but significant binding potential on estrogen receptors and are therefore considered estrogenic.

When evaluating phase 1 metabolism, it is important to look at the ratios of these three metabolites in the pie chart and population sliders to see which pathways are metabolically preferred, see **Figure 5.2**.

2-HYDROXY-ESTRONE (2-OH-E1)

CYP-1a1 is the enzyme responsible for the metabolism of E1 to 2-OH-E1 and E2 to 2-OH-E2. This is the preferred pathway because 2-OH-E1 is the most stable of the three phase 1 estrogen metabolites measured on the DUTCH Test. The 2-OH metabolites have the weakest binding potential to the estrogen receptor when compared to the 4-OH and 16-OH-E1 metabolites.

Like the 4-OH estrogen metabolites, the 2-OH metabolites have the potential to become reactive quinones that bind to DNA and cause DNA damage, especially if they are not adequately methylated. However, unlike the 4-OH estrogen metabolites, when 2-OH metabolites bind to DNA they create stable DNA adducts instead of more damaging (unstable) depurating adducts. Overall, it's best if 2-OH-E1 and 2-OH-E2 are within range.

Potential Support Considerations if Low

If the 2-OH-E1 preference is low, consider supporting CYP-1a1 activity and the preferred pathway with cruciferous vegetables, rosemary, ground flaxseeds, organic non-GMO soy, fish oil, and DIM/I3C[66]. Trying to upregulate the 2-OH pathway when it is not low is not necessarily recommended. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Potential Support Considerations if Elevated

If the 2-OH-E1 dial is high or the 2-OH pie chart percentage is above 80%, consider supporting phase 2 and phase 3 estrogen detoxification and lowering estrogen if elevated. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

4-HYDROXY-ESTRONE (4-OH-E1)

CYP-1b1 is the enzyme responsible for E1 metabolism to 4-OH-E1 and E2 to 4-OH-E2. This pathway is considered the most genotoxic as the 4-OH estrogen metabolites can become reactive quinones that have the potential to bind to DNA and form damaging (unstable) depurating adducts. 4-OH estrogen metabolites may increase breast, endometrial, and prostate cancer risk.

Potential Support Considerations if Elevated

If the 4-OH-E1 dial is high or the pie chart 4-OH percentage is above 11%, consider assessing for CYP-1b1 inducers[67], lowering estrogen if elevated, supporting CYP-1a1 and the 2-OH preferred pathway, supporting phase 2 and phase 3 estrogen detoxification, and supporting glutathione and antioxidants to help neutralize the reactive quinones and potentially reduce the downstream DNA damage. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

16-HYDROXY-ESTRONE (16-OH-E1)

CYP-3a4 is the enzyme responsible for E1 metabolism to 16-OH-E1, and E2 to 16-OH-E2 (estriol). 16-OH-E1 is a proliferative estrogen, although less estrogenic than estradiol. The 16-OH-E1 metabolites bind most strongly to the estrogen receptor when compared to the 2-OH and 4-OH metabolites, however, its binding potential is still considered weak when compared to E2.

Elevations in 16-OH-E1 may exacerbate estrogen excess symptoms and can contribute to estrogen sensitive tissue proliferation (breast, endometrial, prostate, etc.). 16-OH-E2 (also known as E3, or estriol) is a safer estrogen metabolite, as it is a very weak estrogen and may have protective properties. Both 16-OH-E1 and 16-OH-E2 are important for bone health.

Potential Support Considerations if Low

If the 16-OH-E1 dial is low consider supporting bone health, as a high 2-OH/16-OH ratio may be less beneficial for bone mineral density. If E2 is within the expected range, then there may be less concern for a lower 16-OH-E1, as E2 is a more potent estrogen than 16-OH-E1 and has the greatest bone-protective effects. Assess for CYP-3a4 inhibitors as these may decrease 16-OH-E1[68] metabolism, and consider supporting estrogen, if appropriate. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Potential Support Considerations if Elevated

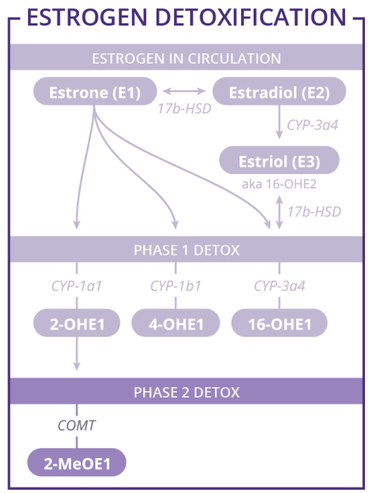
If the 16-OH-E1 dial is high or the 16-OH-E1 pie chart percentage is above 30%, consider assessing for CYP-3a4 inducers[69], lowering estrogen if elevated, supporting CYP-1a1 and the 2-OH preferred pathway, and supporting phase 2 and phase 3 estrogen detoxification. 16-OH-E1 gets glucuronidated and sulfated in phase 2, see **"Appendix A: DUTCH Test Patterns" on page 148** and **"Appendix B: Potential Support Considerations" on page 149**, for more information.

NOTE

> *CYP-3a4 is a major detoxification enzyme in the body and is involved in the metabolism of many pharmaceutical medications, so use caution if inhibiting its enzymatic activity.*

PHASE 2 DETOX

Figure 5.3: Phase 2 Detox



After phase 1, estrogens go through further transformation through phase 2 detox, where the DUTCH Test measures 2-methoxy-E1.

OVERVIEW

After phase 1, estrogens go through further transformation through phase 2 detox, see **Figure 5.3**. Many phase 1 estrogen metabolites have the potential to cause oxidative damage. Therefore, it's important that the 2-OH and 4-OH catechol estrogens (phase 1 estrogen metabolism products) are efficiently and adequately methylated, sulfated, and glucuronidated in phase 2 and inactivated into more stable, water-soluble compounds like 2-Me-E1.

The DUTCH Test measures the phase 2 estrogen metabolite, 2-methoxy-E1. Phase 2 methylation activity is measured by comparing the relative amount of 2-OH-E1 to 2-Me-E1 in the slider, see **Figure 5.4**. If urinary phase 1 catechol estrogen metabolites are more than double the methylated metabolites (e.g., if 2-OH-E1 is more than double 2-methoxy-E1), this may indicate sluggish methylation.

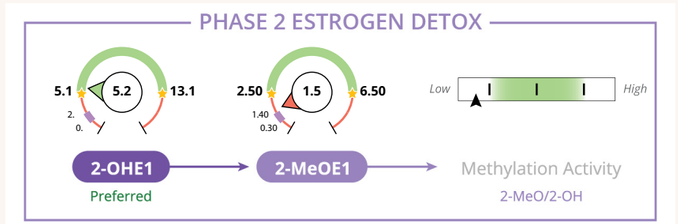


Figure 5.4: *Methylation Activity Population Slider*
Phase 2 methylation activity is measured by comparing the relative amount of 2-OH-E1 to 2-Me-E1 in the population slider.

2-METHOXY-ESTRONE (2-Me-E1)

Methylation of the 2-OH and 4-OH catechol estrogens in phase 2 is carried out by the COMT (catechol-o-methyltransferase) enzyme. COMT helps metabolize catechols through the liver[70].

Adequate methylation activity is important for converting reactive phase 1 metabolites into water-soluble, inactive estrogen metabolites that can no longer damage DNA.

The methylation activity slider shows the person's ratio of 2-Me-E1 to 2-OH-E1. The DUTCH Test provides insight into catechol estrogen methylation activity. The methylation gauge does not measure whole body methylation.

We often see that those with COMT SNPs tend to have lower methylation activity reflected on DUTCH testing.

If estrogen methylation is sluggish, then the 2-OH and 4-OH catechol estrogens can become oxidized to form 2- and 4- estrogen reactive quinones. These quinones, especially the 4-OH quinones, can damage DNA and increase cancer risk.

NOTE

If the 2-Me-E1 dial is high: The 2- and 4-methoxy estrogens appear to have anti-cancer benefits, so an elevated 2-methoxy-E1 itself may not be problematic. In contrast, elevated phase 1 metabolites can be problematic and should be assessed.

Methylation Activity is Low

This indicates sluggish 2-OH-E1 methylation. Sluggish methylation can be due to genetics (COMT, MTHFR, etc.)[71], epigenetics (environmental or lifestyle factors that affect gene expression), decreased precursors, and factors that may inhibit methylation, such as estrogen dominance, gut infections, quercetin, green tea, PCBs, BPA, heavy metals, and nutrient deficiencies.

The rate-limiting nutrients for COMT include vitamin B6 and magnesium. Deficiencies in folate or vitamin B6, B12, glycine, choline, or methionine can cause decreased methyl donors. Consider testing genetic SNPs associated with COMT and MTHFR. See the **"Appendix B: Potential Support Considerations" on** **page 149** for more information on how to support methylation.

Methylation Activity is Elevated

May suggest quick function of the COMT enzyme for estrogen metabolism. This is much less common than under-methylation and may be due to high intake of methylation supplements or a fast COMT, which can be genetic.

Methylation Activity is Within Range

This indicates that methylation of 2-OH-E1 is not sluggish. In some instances, supporting methylation may still be appropriate (for example, in cases of estrogen excess).

OVERVIEW

Phase 3 of estrogen detoxification describes the excretion of estrogen from the body via bile, stool, and urine. The hormones from phase 2 go through phase 3 estrogen detoxification, where they are eliminated in the bile (via stool) and urine. It is important that phase 3 is functioning optimally.

The DUTCH Test does not include any direct phase 3 estrogen detoxification markers. Measuring the enzyme beta-glucuronidase in stool may be helpful if estrogen is elevated, as elevations in this enzyme can result in estrogens being reabsorbed from the stool and recirculated in the body.

Indican

Indican is a gastrointestinal tract dysbiosis marker, see **“Indican” on page 139** for more information. Elevated indican may identify people with dysbiosis, which may impact estrogen levels. Consider supporting phase 3 by ensuring adequate bowel movements, hydration, fiber, calcium-d-glucarate, and stress reduction.

66 DIM and indole-3-carbinol (I3C) are sometimes used to induce CYP-1a1 (the preferred pathway towards 2-OH), however be mindful that they also tend to lower E1 and E2 (Newman, M., Smeaton, J. Exploring the impact of 3,3’-diindolylmethane on the urinary estrogen profile of premenopausal women. BMC Complement Med Ther 24, 405 (2024).) which may not be appropriate for every person. When using DIM and/or I3C, it is important that phase 2 detoxification is well supported so that the phase 1 metabolites do not build up in the body and cause oxidative damage. If on estrogen replacement therapy (ERT), sometimes a relatively higher dose of estrogen may be needed when used in combination with estrogen-lowering compounds such as DIM and/or I3C to achieve desired clinical outcomes. If on Tamoxifen, note that DIM and I3C may render Tamoxifen less effective.

67 CYP-1b1 inducers include inflammation, polycyclic aromatic hydrocarbons (PAHs), xenoestrogens, alcohol, and smoking. There may also be a genetic predisposition for faster CYP-1b1 activity.

68 CYP-3a4 inhibitors include resveratrol, peppermint, rosemary, wild yam, CBD, grapefruit, and various medications. There may also be a genetic predisposition for slower CYP-3a4 activity.

69 CYP-3a4 inducers include St. John’s wort, pesticides, caffeine, excess omega-6 fatty acid consumption, inflammatory cytokines (especially in the gut), smoking, polycyclic aromatic hydrocarbons (PAHs), alcohol, obesity, and hypothyroidism. There may also be a genetic predisposition for faster CYP-3a4 activity.

70 Catechols include 2-OH and 4-OH estrone and estradiol metabolites, dopamine, norepinephrine and epinephrine, coffee, dark chocolate, and green tea.

71 Genetic SNPs in MTHFD1, SHMT1, MTHFR, MTR, MTRR, BHMT, COMT, FUT2 and TCN2 may affect methylation capacity. Note that genetic SNPs in COMT that slow COMT activity can slow methylation activity even in the presence of adequate methyl donors.

SECTION SIX

Cortisol

Introduction

Cortisol research is ever changing. The information presented below is extrapolated from the data and research completed at DUTCH and represents our best understanding and interpretation of the existing data on cortisol and its metabolism. We appreciate your understanding as we continue to reach for excellence in updating our resources.

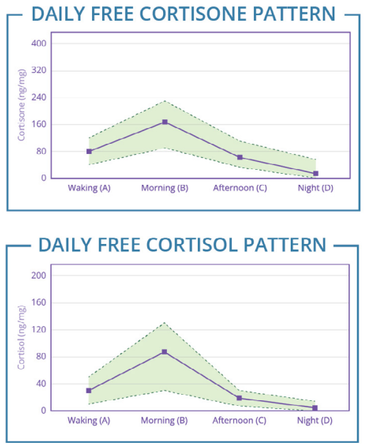
Cortisol is an essential hormone that is released by the adrenal cortex zona fasciculata in small amounts on a daily basis in a circadian rhythm. Cortisol is released in larger amounts in response to stress. Cortisol, a glucocorticoid, helps control blood sugar levels, regulates mood and metabolism, reduces inflammation, and assists with memory formation.

Cortisol production is governed by the HPA axis. The HPA axis is the communication between the hypothalamus and pituitary gland in the brain and the adrenal glands located on the tops of the 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). ACTH signals the adrenal glands to release cortisol, DHEA, DHEA-S, and androstenedione. Assessing these hormones on the DUTCH Test can give insight into HPA axis function and health.

LET'S GET STARTED ▶▶

EVALUATING CORTISOL

Figure 6.1: Measuring Cortisol



The DUTCH Test measures the diurnal patterns of cortisol and cortisone throughout the day.

OVERVIEW

Cortisol should have a diurnal pattern that is highest in the morning and lowest at night before bedtime. In most instances, daily patterns of free cortisol and free cortisone will be substantially similar, see **Figure 6.1**.

NOTE

> *Patients are instructed to collect on a typical day to best capture their typical cortisol pattern. Abnormal results should be considered along with the person's symptoms and any unusual occurrences of the day.*

In some patients, such as patients with exposure to topical hyrdocortisone cream, cortisol can be significantly elevated, and cortisone daily patterns provide a more accurate representation of HPA axis function and may be used to assess diurnal pattern. See **"Cortisol (Hydrocortisone Cream) Contamination" on page 124** for more information.

USING THE DUTCH TEST

Total Free Cortisol

We can assess cortisol's overall tissue availability by looking at the 24-hour free cortisol and cortisone, see **"Total Free Cortisol" on page 120** for more information.

Daily Free Cortisol & Cortisone Patterns

We can estimate HPA axis function/dysfunction by looking at the free cortisol and free cortisone daily patterns, see **"The Cortisone Shadow" on page 122** for more information.

Metabolized Cortisol

We can estimate the total adrenal cortisol production by assessing metabolized cortisol, see **"Metabolized Cortisol" on page 125** for more information.

Cortisol Clearance Rate

We can estimate the cortisol clearance rate by comparing the 24-hour free cortisol and cortisone with metabolized cortisol, see **"Cortisol Clearance Rate" on page 126** for more information.

THE vs THF Preference

We can estimate the systemic preference for (inactive) cortisone or (active) cortisol by looking at the DUTCH Test THE vs. THF slider, see **"THE vs THF" on page 128** for more information.

SIGNS & SYMPTOMS OF LOW CORTISOL

Low cortisol may be associated with fatigue, burnout, low mood, low motivation, low libido, sleep apnea, orthostatic hypotension, feeling dizzy/weak, and fainting.

Other potential causes for these symptoms include:

- Fatigue/Burnout: Low testosterone, sleep dysregulation, lifestyle/diet choices, infection, autoimmunity, blood sugar dysregulation, nutrient deficiency, neurotransmitter issues, thyroid issues, electrolyte imbalance, high histamine.
- Low Mood/Low Motivation: Neurotransmitter issues, thyroid issues, nutrient deficiency, low or high estrogen, low testosterone, low DHEA.
- Low Libido: Low testosterone, low DHEA, low estrogen, sleep dysregulation, neurotransmitter issues, thyroid issues.
- Sleep Apnea: Overweight, head/neck anatomy, infection (especially sinus), high histamine.
- Orthostatic Hypotension: Dehydration, nutrient deficiency, electrolyte imbalance, POTS, blood sugar dysregulation.
- Feeling Dizzy/Weak/Fainting: Dehydration, nutrient deficiency, electrolyte imbalance, infection, sleep dysregulation, blood sugar dysregulation.

See **"Appendix B: Potential Support Considerations" on page 149** for help targeting these potential root causes.

SIGNS & SYMPTOMS OF ELEVATED CORTISOL

Elevated cortisol may be associated with anxiety, depression, panic attacks, insomnia, weight gain (belly fat), brain fog, inflammation, pain, insulin/blood sugar dysregulation, high blood pressure, and hair loss.

Other potential causes for these symptoms include:

- Anxiety/Depression/Panic Attacks: High estrogen, low progesterone, neurotransmitter issues, thyroid issues, sleep dysregulation, blood sugar dysregulation, nutrient deficiency.
- Insomnia: Blood sugar dysregulation, nighttime blue light exposure, caffeine or alcohol before bed, thyroid issues, gut dysbiosis, low progesterone, low melatonin.
- Weight Gain (Belly Fat): Lifestyle/diet choices, low testosterone, low DHEA, high estrogen, hypothyroidism, blood sugar dysregulation, sleep dysregulation.
- Brain Fog: Low estrogen, nutrient deficiency, neurotransmitter issues, thyroid issues, blood sugar dysregulation.
- Inflammation/Pain: Lifestyle/diet choices, lack of exercise, nutrient deficiency.
- Insulin/Blood Sugar Dysregulation: Lifestyle/diet choices, lack of exercise, nutrient deficiency.
- High Blood Pressure: Kidney disease, nutrient deficiency, cardiovascular issues, age, tobacco use, diabetes, overweight/obesity, lifestyle/diet changes.
- Hair Loss: Thyroid issues, iron deficiency, endocrine disruptors, nutrient deficiency, high androgen (testosterone or DHT).

See **"Appendix B: Potential Support Considerations" on page 149** for help targeting these potential root causes.

TOTAL FREE CORTISOL

OVERVIEW

Total free cortisol[72] is the best marker to assess overall tissue exposure to cortisol. It is calculated by adding up the four urinary (or five salivary) free cortisol points on the graph. It correlates well with a true 24-hour urine free cortisol; however, it does not consider transitory shifts in cortisol in the late morning and early afternoon.

When considering treatments that affect the entire HPA axis, also look at the metabolized cortisol to get an idea of total cortisol production. Total free cortisol and metabolized cortisol levels don't always agree due to the differences in the cortisol clearance rate. For example, if a person has a fast clearance rate, then their free cortisol may be low while their metabolized cortisol may be above range. Keep in mind that free cortisol only represents about 5% of the total circulating cortisol. The other 95% is bound to cortisol binding globulin (80-90%) and albumin (10%).

NOTE

> *If the total free cortisone dial points higher than the total free cortisol dial, then the free cortisol in circulation may not be as low as the free cortisol graph implies. See* **"Special Cases" on page 121** *and* **"The Cortisone Shadow" on page 122***, for more information.*

When the term "total free cortisol" is used, it is referencing the 24-hour free cortisol for urinary results (DUTCH Complete, Adrenal panel) or the "total salivary cortisol" for salivary results (DUTCH Plus, DUTCH CAR):

- Total free cortisol (urine) = 24-hour free cortisol
- Total free cortisol (saliva) = total salivary cortisol

SIGNS, SYMPTOMS, & ASSOCIATED CONDITIONS

See **"Appendix B: Potential Support Considerations" on page 149** for help targeting these potential root causes.

Free Cortisol is Low

Low free cortisol may be associated with fatigue, burnout, low mood, low motivation, low libido, dizziness, weakness, fainting, sleep apnea, orthostatic hypotension, traumatic brain injuries (TBI), concussions, and PTSD. Elevated renal glomerular filtration rate (GFR) can lead to falsely low free cortisol in the urine; however, it does not affect the metabolized cortisol value.

Free Cortisol is Elevated

High free cortisol may be associated with stress, anxiety, panic attacks, depression, insomnia, weight gain (belly fat), brain fog, blood sugar dysregulation, inflammation, pain, high blood pressure, hair loss, and immune suppression. Low urinary creatinine can elevate urinary free cortisol[73]. Consider checking in with the person regarding the events that took place during collection day, as cortisol is an acute stress response hormone and results could reflect a high stress state that may not be a daily occurrence. Cortisol can elevate due to low blood sugar, anticipatory stress, anxiety, stressful events, acute inflammation, acute pain, intense exercise and caffeine intake.

Free Cortisol is Within Range

A normal free cortisol reflects a non-stressful environment when testing.

A normal free cortisol may be associated with a healthy blood pressure, blood sugar regulation, reduced inflammation, and improved mood, energy, motivation, focus, and sleep.

SPECIAL CASES

Free Cortisone is Higher Than Free Cortisol

The enzyme 11b-HSD2 deactivates cortisol to cortisone locally in the kidneys and salivary glands. When the activity of this enzyme is increased, more cortisol will show up in the urine or saliva as cortisone. Therefore, if the total free cortisone dial points higher than the total free cortisol dial, then the free cortisol in circulation may not be as low as the free cortisol graph implies.

Free Cortisol is Higher Than Free Cortisone

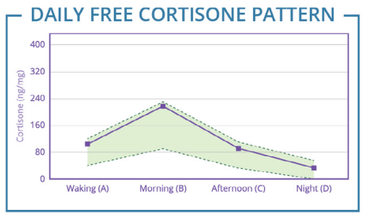
A free cortisol that is higher than free cortisone suggests an acute stressor when testing and may be associated with acute migraines, high fever, psychological stressor, or injury, to name a few.

However, a free cortisol that is higher than free cortisone may be due to hydrocortisone contamination, see **Figure 6.2**. Hydrocortisone contamination only affects free cortisol, not free cortisone. Despite its name, hydrocortisone is active cortisol. In this setting, use of free cortisone to estimate tissue availability may better represent the diurnal cortisol pattern, see **"Cortisol (Hydrocortisone Cream) Contamination" on page 124** for more information.

Figure 6.2: ***Hydrocortisone Contamination***
A free cortisol that is much higher than a free cortisone may be due to hydrocortisone contamination.

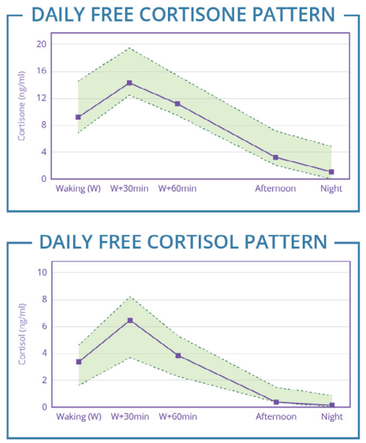
THE CORTISONE SHADOW

Figure 6.3: Free Cortisol and Free Cortisone's Diurnal Pattern in Urine



Both free cortisol and free cortisone have a diurnal pattern in the urine.

Figure 6.4: Free Cortisol and Free Cortisone's Diurnal Pattern in Saliva



Both free cortisol and free cortisone have a diurnal pattern in the saliva.

OVERVIEW

There are two graphs on the DUTCH adrenal page: one shows the diurnal pattern of free cortisol whereas the other shows the diurnal pattern of free cortisone.

Most clinicians are familiar with reading the first pattern (free cortisol only) and are not sure what to do with the free cortisone graph, especially when the two diurnal patterns don't match. Please read through this section and look at the images to learn about the significance and importance of using the free cortisone pattern to get a better understanding of free cortisol levels in circulation.

Measuring Cortisone

Cortisone is the inactive form of cortisol and is made directly from cortisol in the kidneys, colon, and saliva glands. (active --> inactive) It is then converted back to cortisol in the liver, fat, etc. (inactive --> active).

Cortisone does not have a diurnal pattern in the serum [q], but it does in urine and saliva, see **Figure 6.3** and **Figure 6.4**.

Free cortisone in the urine and saliva reflects the free cortisol that entered the kidneys or the saliva glands and was converted to cortisone locally before excretion. This makes free cortisone a secondary, confirmatory marker for the up and down diurnal pattern of free cortisol.

NOTE

Studies have found salivary free cortisone to correlate to serum free cortisol, better than salivary free cortisol. [r][s]

Some benefits of testing cortisone include:

- It helps to confirm the diurnal pattern of cortisol.
- Sometimes it provides more clinically useful information than the free cortisol pattern (examples later in this section).
- It helps to identify if there is potential cortisol contamination from medications like topical eczema creams that contain hydrocortisone (bioidentical cortisol).

[q] Walker BR, et al. Mineralocorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. Clin Endocrinol (Oxf). 1992 ;37(6):483-492.

[r] Blair J, et al. Salivary cortisol and cortisone in the clinical setting. Curr Opin Endocrinol Diabetes Obes. 2017; 24(3):161-168.

[s] Perogamvros I, et al. Salivary Cortisone Is a Potential Biomarker for Serum Free Cortisol. J Clin Endocrinol Met. 2010; 95(11):4951-4958.

HOW TO READ THE FREE CORTISONE PATTERN

First, keep in mind that the goal is to understand the free cortisol pattern in circulation. This is best measured in the serum (via serial blood draws) however this method of measurement is very impractical and for many people so stressful that saliva and urine measurements are more accurate. Fortunately, salivary and urinary cortisone reflect systemic free cortisol.

PATTERN EXAMPLES

Both Free Cortisol & Cortisone Match

When the free cortisol and free cortisone patterns in the urine or saliva match (both high, both low, both normal), cortisone confirms cortisol. In **Figure 6.5**, cortisone and cortisol follow the same pattern. High cortisol in the afternoon and evening is confirmed by high cortisone in the afternoon and evening. Similarly, in **Figure 6.6**, low/flat cortisol throughout the day is confirmed by low/flat cortisone throughout the day.

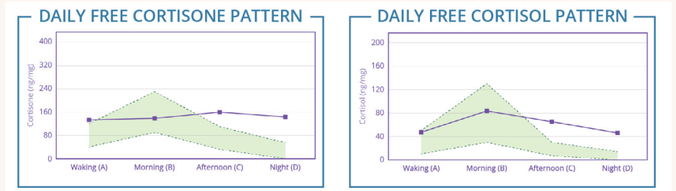


Figure 6.5: *Free Cortisol and Free Cortisone Match*
In this example the free cortisone pattern confirms the elevated afternoon and evening free cortisol.

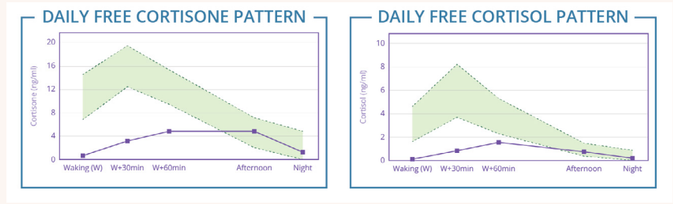


Figure 6.6: *Free Cortisol and Free Cortisone are Both Low*
In this example, the low free cortisone pattern confirms low free cortisol.

Free Cortisol is Elevated but Cortisone is Not

When free cortisol in the urine or saliva is high but cortisone is not (free cortisone is normal or low), then free cortisol levels may not be quite as high in circulation (serum) as the cortisol levels in the urine or saliva imply.

NOTE

This pattern with elevated free cortisol could be due to hydrocortisone contamination, see **"Cortisol (Hydrocortisone Cream) Contamination" on page 124** *for more information.*

Free Cortisol is Low, but Cortisone is Not

When free cortisol in the urine or saliva is low but free cortisone is not (free cortisone is normal or elevated), free cortisol levels may not be quite as low in circulation (serum) as the cortisol levels in the urine or saliva imply and this should influence your approach to symptoms.

In **Figure 6.7**, the free cortisol is lower in the reference range than the free cortisone at each point on the graph. Since free cortisone comes from free cortisol, free cortisone may reflect serum free cortisol better in this case. For unknown reasons, this person's kidneys deactivate cortisol into cortisone more than most. If only free cortisol was tested, this person might be incorrectly treated for very low cortisol. Instead, this person's total free cortisol in circulation is likely on the higher end, as their urine free cortisone total is above range.

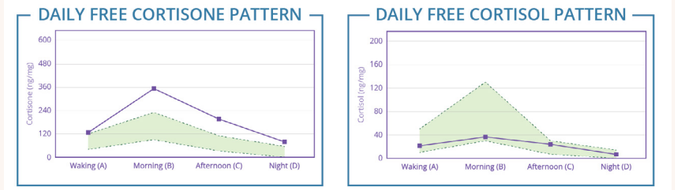


Figure 6.7: ***Free Cortisol is Low but Free Cortisone is Not***
If only free cortisol was tested, this person might be incorrectly treated for very low cortisol.

CORTISOL (HYDROCORTISONE CREAM) CONTAMINATION

Despite its name, hydrocortisone is actually bioidentical active cortisol. When a person touches the sample paper with hydrocortisone cream on their hands (even a little), it can contaminate the sample paper.

Cortisol must enter the body in order to be deactivated to cortisone, thus when the sample paper is contaminated by hydrocortisone cream, the free cortisol will be affected but the free cortisone won't. In these cases, the free cortisol pattern can be ignored, and the free cortisone pattern is more accurate.

In **Figure 6.8**, the patient contaminated their bedtime sample with hydrocortisone cream. Hydrocortisone cream contamination affects the free cortisol graph only, thus the free cortisone graph is a better representation of their true free cortisol diurnal pattern.

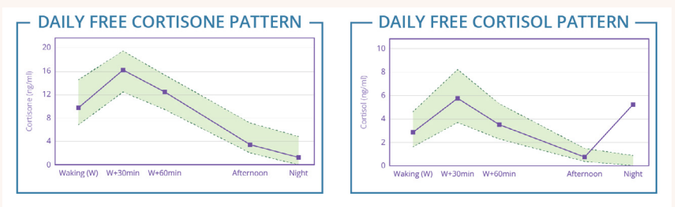


Figure 6.8: ***Hydrocortisone Contamination in the Saliva***
A free cortisol that is much higher than a free cortisone may be due to hydrocortisone contamination.

METABOLIZED CORTISOL

OVERVIEW

Metabolized cortisol is the sum of a-THF, b-THF, and b-THE (the most abundant cortisol metabolites), see **Figure 6.9**. Metabolized cortisol may be the best marker for assessing total adrenal cortisol production. Remember, this is influenced by HPA axis signaling and cortisol clearance rates.

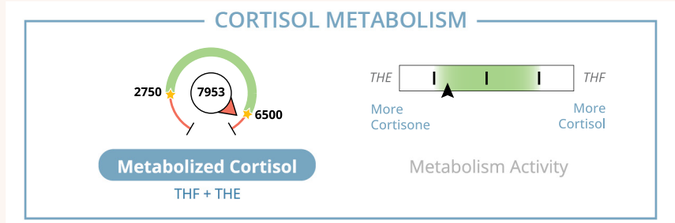


Figure 6.9: ***Metabolized Cortisol (Total Cortisol Production)***
Metabolized cortisol is the sum of the cortisol metabolites a-THF, b-THF, and b-THE, and may be the best marker for assessing total adrenal cortisol production.

SIGNS, SYMPTOMS, & ASSOCIATED CONDITIONS

See **"Appendix B: Potential Support Considerations" on page 149** for help targeting these potential root causes.

Metabolized Cortisol is Low

Total cortisol production may be low. Low cortisol production may be associated with long-term stress, poor sleep hygiene, untreated chronic sleep apnea, pituitary or hypothalamic dysfunction, and certain medications known to suppress HPA axis activity[74]. Less common associations may include TBI, NCAH, CAH, and Addison's disease[75].

Metabolized Cortisol is High

Total cortisol production may be high. High cortisol production may be associated with stress, anxiety, acute inflammation, infection, pain, caffeine, blood sugar dysregulation, Cushing's syndrome[76], and oral hydrocortisone supplementation[77].

Metabolized Cortisol is Within Range

Total cortisol production appears appropriate for the clinical situation[78]. Appropriate, for the clinical situation, cortisol metabolism and clearance does not rule out conditions that may result in low or high metabolized cortisol, as outlined above.

CORTISOL CLEARANCE RATE

OVERVIEW

We can estimate the cortisol clearance rate (CCR) by comparing the total free cortisol[79] with the metabolized cortisol.

CCR IS SLOW: METABOLIZED CORTISOL < FREE CORTISOL

Potential Root Causes

This hypometabolic pattern may indicate that cortisol clearance rate is slow, **Figure 6.10**. Slow cortisol metabolism and clearance may be associated with things that tend to lower the overall metabolic rate, such as hypothyroidism (or insufficient thyroid medication), low calorie intake (anorexia), low bile acid, and poor liver and mitochondrial function.

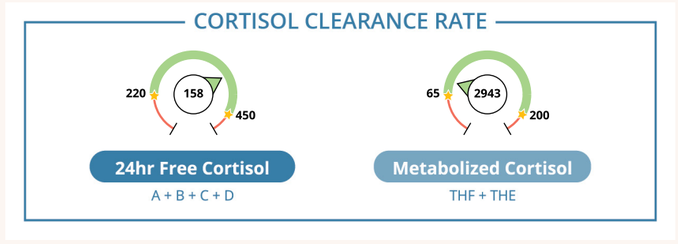


Figure 6.10: ***Cortisol Clearance Rate is Slow***
When metabolized cortisol is relatively lower than free cortisol, it may indicate that the cortisol clearance rate is slow.

Potential Support Considerations

In addition to treating the underlying cause, other evaluations and support considerations may include ordering a full thyroid panel[80], ensuring sufficient caloric intake, supporting liver function, and supporting mitochondrial function. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

CCR IS FAST: METABOLIZED CORTISOL > FREE CORTISOL

Potential Root Causes

This hypermetabolic pattern may indicate that the cortisol clearance rate is fast, see **Figure 6.11**. Fast cortisol metabolism and clearance may be associated with obesity, inflammation, blood sugar and insulin dysregulation, hyperthyroidism (or excessive thyroid medication), and long-term stress. In obesity, adipose tissue sequesters cortisol, which in turn may increase cortisol production (metabolized cortisol).

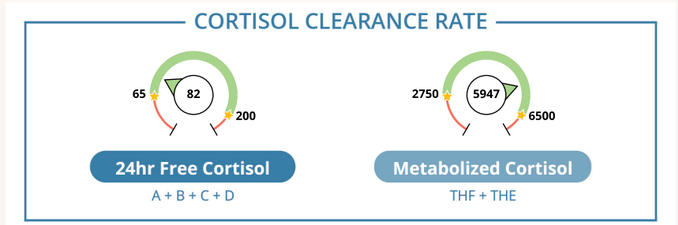


Figure 6.11: ***Cortisol Clearance Rate is Fast***
When metabolized cortisol is relatively higher than free cortisol, it may indicate that the cortisol clearance rate is fast.

Potential Support Considerations

In addition to treating the underlying cause, other evaluations and support considerations may include ordering a full thyroid panel[81], weight loss if appropriate, reducing inflammation, regulating blood sugar and insulin, reducing stress, which may include supporting the parasympathetic nervous system (PNS), providing herbal calming support and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

> *When metabolized cortisol is high but free cortisol and cortisone are low, cortisol levels may be different depending on the location within the body. For example, levels of cortisol may be elevated in the adrenal glands and may result in increased conversion of noradrenaline to adrenaline. Oppositely, levels of cortisol may be low in the brain and may result in increased ACTH secretion. Therefore, use caution with efforts aimed at increasing HPA axis activity as these may exacerbate some symptoms.*

CCR IS NORMAL: METABOLIZED CORTISOL = FREE CORTISOL

This pattern may indicate that the cortisol clearance rate is as expected for the clinical situation, see **Figure 6.12**. Normal cortisol metabolism and clearance does not rule out conditions that may slow down or speed up cortisol metabolism, as outlined above.

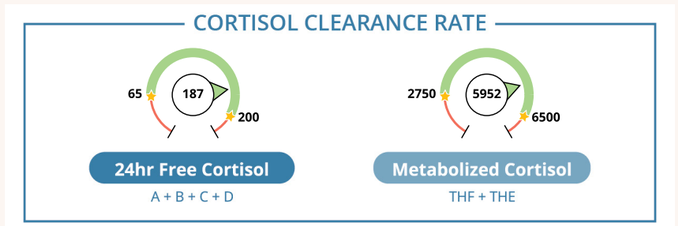


Figure 6.12: ***Cortisol Clearance Rate is Normal***
When metabolized cortisol and free cortisol match (point in the same direction), it may indicate that the cortisol clearance rate is as expected for the clinical situation.

THE VS THF

Figure 6.13:
Systemic Preference Population Slider

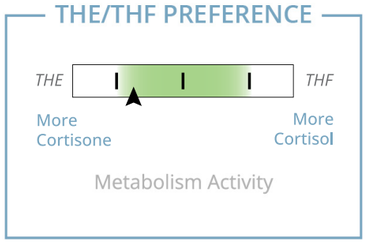


Figure 6.14:The More Cortisone Metabolites (THE) vs More Cortisol Metabolites (THF) slider on the DUTCH Test is the best way to assess systemic preference for inactive cortisone or active cortisol, respectively.

ESTIMATING SYSTEMIC[82] PREFERENCE

The More Cortisone Metabolites (THE) vs More Cortisol Metabolites (THF) slider on the DUTCH Test, see **Figure 6.13**, is the best way to assess systemic preference for inactive cortisone or active cortisol, respectively. Free cortisone is metabolized into b-tetrahydrocortisone (b-THE) by 5b-reductase. Free cortisol is metabolized into a-tetrahydrocortisol (a-THF) by 5a-reductase and b-tetrahydrocortisol (b-THF) by 5b-reductase. Cortisone's THE metabolites and cortisol's THF metabolites are inactive and cannot be reactivated.

THE PREFERENCE

A person may have a THE preference if the 11b-HSD2 enzyme is readily deactivating cortisol to cortisone in the kidneys, saliva glands and colon.

Signs & Symptoms

A THE preference may be seen with chronic stress, elevated estrogen levels, glucocorticoid medications, hyperthyroidism (or too much thyroid medication) and during the end stage of infection. With hyperthyroidism (or too much thyroid medication), the metabolized cortisol dial tends to point relatively higher than the total free cortisol (and cortisone) dials, as cortisol metabolism and clearance tends to speed up in the presence of increased thyroid activity.

Potential Support Considerations

In addition to treating the underlying cause, other evaluations and considerations may include assessing for chronic adrenal stressors[83], PNS calming support, and licorice root[84]. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

THF PREFERENCE

A person may have a THF preference if the 11b-HSD1 enzyme is readily reactivating cortisone to cortisol in the liver, fat cells and the periphery.

Signs & Symptoms

A THF preference may be seen with obesity, insulin resistance, inflammation, licorice root supplementation, hypothyroidism (or insufficient thyroid medication), and excessive exposure to, or difficulty detoxifying, specific environmental chemicals such as phthalates, organotoxins, and alkylphenols. With hypothyroidism (or not enough thyroid medication), the metabolized cortisol dial tends to point lower than the total free cortisol (and cortisone) dials, as cortisol metabolism and clearance may be slow.

Potential Support Considerations

In addition to treating the underlying cause, other evaluations and considerations may include weight loss if appropriate, regulating blood sugar and insulin levels, reducing inflammation, stress reduction, which may include PNS support, herbal calming support, and GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

CORTISOL AWAKENING RESPONSE

OVERVIEW

We can estimate a person's ability to cope with stress by looking at the cortisol awakening response (CAR) in saliva, see **Figure 6.15**. The CAR may be viewed as a HPA axis resiliency marker, an anticipatory marker of upcoming daily events, and a surrogate marker for HPA axis plasticity/reactivity. The CAR is influenced by several factors including gender, health status, and stress perception.

The CAR is relatively distinct from earlier and later components of circadian cortisol secretion. Studies document that the CAR was found to be unrelated to cortisol levels during the remainder of the day. It is important to remember that the CAR is neither a general HPA axis marker, nor is secreted in response to stress. [t]

Therefore, the CAR and remainder of the diurnal curve should be interpreted separately and then assimilated to determine the presence and severity of HPA axis dysfunction, resiliency, and adaptability. An individual can have a normal CAR, while the remainder of the diurnal pattern may be high, within the reference range, low-normal, or low.

MEASURING THE CAR

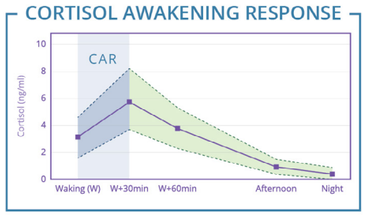
The CAR is measured as the percent difference between the waking and 30-minute free cortisol. In healthy adults (assuming the test is timed correctly) the magnitude of the CAR was found to range between 50-160% or a rise of 1.5-4.0ng/mL. The starting point is the sensation of awakening (S1), then the cortisol increases after awakening (CAR), and then the +30-minute sample (S2). In healthy adults, the +60-minute sample should approximate S1. [u]

An increased CAR has been associated with relapsing-remitting multiple sclerosis, visceral adiposity, metabolic syndrome in females, etc. Conversely, a decreased CAR has been associated with type 2 diabetes, chronic fatigue syndrome, hypertension, and functional gastrointestinal disorders, e.g., irritable bowel syndrome. [v]

Free Cortisone and the CAR

There is no literature assessing cortisone in place of cortisol for assessing the CAR. However, it is not unreasonable to take the cortisone "sense of awakening" sample S1 and the +30 sample into consideration when determining the best overall treatment plan.

Figure 6.15: Daily Free Cortisol with Cortisol Awakening Response (CAR)



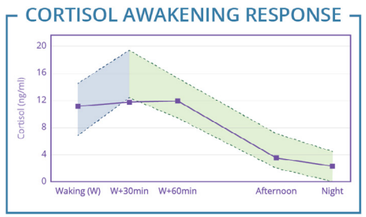
Measuring cortisol can provide valuable insights into hypothalamic-pituitary-adrenal (HPA) axis health.

[t] Stadler T, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinology. 2016; 63: 414-432.

[u] Fries E, et al. The cortisol awakening response (CAR): facts and future directions. Int J Psychophysiol. 2009; 72(1): 67-73.

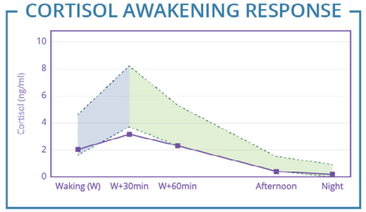
[v] Powell DJ, Schlotz W. Daily Life stress and the cortisol awakening response: testing the anticipation hypothesis. PLoS One. 2012; 7(12): e52067.

Figure 6.16: CAR is Absent



In this example there is no increase in cortisol from waking to +30 minutes after waking.

Figure 6.17: CAR is Low



In this example there is a lower than expected increase in cortisol of 1.12 ng/mL or 55.4% from waking to +30 minutes after waking.

CAR IS ABSENT

Potential Root Causes and Associated Conditions

An absent CAR, see **Figure 6.16**, is seen in people with hippocampal atrophy or damage, especially seen in those with memory loss. It is also seen with high adverse childhood events scores (ACEs), obstructive sleep apnea (OSA), and people with chronic low level noise exposure during sleep, such as those living with high traffic noise.

NOTE

An absent CAR is normal in 15% of adults.

Potential Support Considerations

Addressing the root cause. Research supports the use of cognitive behavioral therapy (CBT) for ACE's and continuous positive airway pressure (CPAP) for people with OSA. Consider improving the sleep sound environment (e.g., with ear plugs). See **"Appendix B: Potential Support Considerations" on page 149** for more information.

CAR IS LOW

Potential Root Causes and Associated Conditions

A low CAR, see **Figure 6.17**, may be associated with type 2 diabetes, chronic fatigue syndrome, hypertension, and functional gastrointestinal disorders, chronic stress or burnout, cardiovascular, autoimmune, atopic, and psychiatric disorders.

Other associations may include awakening in darkness as opposed to light, postpartum depression, major depression, seasonal affective disorder (SAD), PTSD, jet lag insomnia, sleep apnea, and poor sleep quality, and taking medications known to suppress HPA axis activity[85].

Less common associations may include hippocampal and/or pituitary damage and TBI, and retrograde amnesia.

A person may also have a low CAR if their CAR had already occurred prior to sample collection.

A high waking free cortisol may be associated with stress, low blood sugar, sleep apnea, pain, and alcohol consumption the night prior. A high waking cortisol may be associated with a blunted CAR.

Potential Support Considerations

In addition to treating the underlying cause, other considerations may include foundational HPA axis support, sleep hygiene support, rest, therapy (medication) for mental/emotional distress, cognitive support, light exposure upon waking (sun or full spectrum lighting), aerobic exercise, multivitamin (to address possible vitamin deficiencies), CBT during pregnancy and postpartum, SSRIs for depression, CPAP during sleep for OSA, and stress reduction, which may include PNS support, herbal calming support, or GABA support. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

CAR IS ELEVATED

Potential Root Causes and Associated Conditions

An elevated CAR, see **Figure 6.18**, may be associated with job-related stress, anticipatory stress, relapsing-remitting multiple sclerosis, visceral adiposity, metabolic syndrome in females, inflammation, and white light exposure. The data on anxiety and depression are mixed.

NOTE

A person may have a low sense of awakening cortisol point (S1) and a within-range +30-minute point (S2) resulting in a normal or high CAR. This may be a normal response to the day's anticipated events.

A person may have high waking cortisol if their CAR had already begun prior to sample collection.

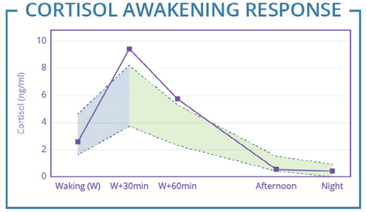
Potential Support Considerations

The key to normalizing an elevated CAR is to address the underlying etiology driving the anticipatory stress. In addition to addressing the above underlying cause(s), other support considerations may include: foundational HPA axis support, PNS support, herbal calming support, GABA support, adaptogenic herbal support, sleep hygiene support, journaling, CBT, CBT for insomnia (CBTI), Rhodiola rosea, and selective serotonin reuptake inhibitors (SSRIs). See **"Appendix B: Potential Support Considerations" on page 149** for more information.

CAR IS WITHIN RANGE

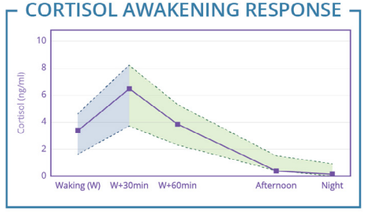
A normal CAR, see **Figure 6.19**, does not rule out conditions that may be associated with a high or low CAR.

Figure 6.18: CAR is Elevated



In this example there is a higher than expected increase in cortisol of 6.9 ng/mL or 269% from waking to +30 minutes after waking.

Figure 6.19: CAR is Within Range



In this example there is an expected increase in cortisol of 3.10 ng/mL or 92.5% from waking to +30 minutes after waking.

ANNOTATIONS

72 Total free cortisol is called “24-hour Free Cortisol” for urine samples and “Total Salivary Cortisol” for salivary samples.

73 Note that the free cortisol and free cortisone data can be somewhat less reliable for samples that have very low creatinine (female <0.15; male <0.2; see page 4 of a DUTCH Plus and DUTCH Complete reports or page 1 of a DUTCH Adrenal Only panel).

74 Medications that are suppressive to the HPA axis include glucocorticoids (prednisone, dexamethasone, fluticasone, budesonide, etc.), opioids, NSAIDs, and tricyclic antidepressants (TCAs). These medications can also suppress adrenal hormone production (such as DHEA, androstenedione, testosterone, and mineralocorticoids). The degree of adrenal suppression is dependent upon type of medication used, strength, dose, frequency of use, duration of use and time since last use. It is not known if natural supplements, such as adaptogens, can overcome adrenal suppression from continued glucocorticoid use.

75 In Addison’s disease, free cortisol and cortisone are very low (approaching zero) with no diurnal pattern and metabolized cortisol is < 1,000ng/mg. Further testing is required for diagnosis. Medication-induced HPA axis suppression (for example, with a glucocorticoid like prednisone) may result in a similar pattern.

76 In Cushing’s disease, free cortisol and cortisone are high throughout the day with no diurnal pattern and metabolized cortisol is elevated. Bedtime cortisol is elevated and often more than four times above the upper reference range limit. Further testing is required for diagnosis. Oral hydrocortisone taken throughout the day may result in a similar pattern.

77 Oral hydrocortisone can lead to elevations in urine free cortisol for 4-6 hours after supplementation and elevations in cortisol metabolites (THE and THF) for 10-12 hours after supplementation. Be aware that cortisol metabolites (THE and THF) will lag behind free cortisol levels, so elevations in cortisol metabolites may be due to supplementation that is no longer affecting free cortisol levels if taken the morning of the test. It is important to know when the person last took their hydrocortisone therapy to properly interpret the results.

78 Be sure to assess the free cortisol and free cortisone levels, as the rate of cortisol clearance (comparing 24-hour free cortisol with metabolized cortisol) can affect metabolized cortisol levels. For example, if total free cortisol is elevated and the metabolized cortisol (cortisol production) is within the normal range or low, this suggests that the rate of cortisol clearance is slow.

79 Note that the total free cortisol dial is called 24 Hour Free Cortisol for urine samples or Total Salivary Cortisol for salivary samples. If the total free cortisone dial points higher than the total free cortisol dial, then the free cortisol in circulation may not be as low as the free cortisol graph implies. See **“The Cortisone Shadow” on page 122** for more information.

80 A full thyroid panel often includes TSH, fT4, fT3, rT3, TPO, and thyroglobulin antibodies.

81 A full thyroid panel often includes TSH, fT4, fT3, rT3, TPO, and thyroglobulin antibodies.

82 Note that it might be helpful to look at the THE vs THF preference and NOT the free cortisol and free cortisone graphs when estimating systemic preference for cortisone and cortisol. This is because the enzyme 11b-HSD2 can affect the local free cortisol and cortisone balance in the kidneys (urine) and salivary glands (saliva).

83 Chronic adrenal stressors may include psychological stress, pain, inflammation, endocrine disrupting chemicals (EDCs), a standard American diet, infection, blood sugar dysregulation, overexercising, overtraining, and poor sleep, etc.

84 Licorice root limits the deactivation of free cortisol into free cortisone and caution is warranted in individuals with hypertension. Licorice root can worsen hypertension. Always monitor blood pressure and electrolyte balance when using licorice root.

85 Medications that are suppressive to the HPA axis include glucocorticoids (prednisone, dexamethasone, fluticasone, budesonide, etc.), opioids, NSAIDs, and tricyclic antidepressants (TCAs). These medications can also suppress adrenal hormone production (such as DHEA, androstenedione, testosterone, and mineralocorticoids). The degree of adrenal suppression is dependent upon type of medication used, strength, dose, frequency of use, duration of use and time since last use. It is not known if natural supplements, such as adaptogens, can overcome adrenal suppression from continued glucocorticoid use.

SECTION SEVEN

Organic Acids

Introduction

Organic acids are metabolic byproducts of cellular activity that may provide insight into nutrient status, inflammation, neurotransmitters, etc. The DUTCH Test measures six nutritional organic acids, three neuro-related markers, and two additional markers. These OATs are measured from the first morning collection.

The DUTCH OATs profile is a combination of biomarkers to complement hormone metabolite measurements including a series of organic acids and other select tests. Organic acids related to nutrient deficiencies include biochemical intermediates that are proven to become elevated in the absence of sufficient nutrient status and other biomarkers may indicate oxidative stress, melatonin deficiency, and/or gut dysbiosis, all of which impact patient hormone stories.

Nutritional Organic Acids

These organic acids act as functional markers of nutrient deficiency. When the body has inadequate cellular levels of vitamin B12, vitamin B6 or glutathione, levels of their corresponding organic acid build up and spill into the urine. In some cases, these markers are more effective than measuring the nutrient directly.

Neurotransmitter Metabolites

These organic acids are the primary metabolite of dopamine, norepinephrine and serotonin. Patients with an imbalance in these neurotransmitters may experience symptoms that are also common with an imbalance in hormones. Hormones (cortisol and estrogen are examples) included in DUTCH testing also directly alter some of these metabolites, so their inclusion provides an even more comprehensive picture of your patient's hormone health.

LET'S GET STARTED

METHYLMALONATE (MMA)

Elevated MMA may be seen in vitamin B12 deficiency. MMA is a metabolic byproduct of the citric acid cycle (Krebs cycle). It is converted to succinyl-CoA by methylmalonyl-CoA mutase (MCM). Adenosylcobalamin is a cofactor for MCM, and deficiency in this form of vitamin B12 can cause MMA to build up in the body.

Vitamin B12 is important for estrogen detoxification (phase 2 methylation), neurotransmitter activity, memory, mood, cognition, energy, gait, and red blood cell production.

Signs & Symptoms

Low vitamin B12 may be associated with fatigue, brain fog, memory problems, muscle weakness, unsteady gait, numbness, tingling, depression, anxiety, shortness of breath, low blood pressure, heart palpitations, migraines, headaches, and low blood pressure.

Contributors to Deficiency

Vitamin B12 deficiency may be associated with low dietary intake, poor absorption, low stomach acid, pernicious anemia, genetic SNPs and/or medications such as metformin, H2 acid blockers, proton pump inhibitors (PPIs), and oral birth control (OCPs).

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Increasing vitamin B12 dietary intake[86]
- Active vitamin B12 supplementation[87]
- Sublingual or injectable vitamin B12 if the individual has low stomach acid while addressing the underlying contributors to low stomach acid[88]
- Genetic SNP testing[89]
- Intrinsic factor antibody testing to rule out pernicious anemia

See **“Appendix B: Potential Support Considerations” on page 149** for more information.

XANTHURENATE & KYNURENATE

Elevated xanthurenate and kynurenate may be seen with vitamin B6 deficiency. Xanthurenate and kynurenate are metabolic byproducts of tryptophan metabolism.

Vitamin B6 is involved in over 100 human enzymatic reactions. Vitamin B6 is important for estrogen detoxification (phase 2 methylation), neurotransmitter production, and healthy glutathione levels.

Signs & Symptoms

Low vitamin B6 may be associated with fatigue, PMS, mood issues, tingling and pain in hands and feet, cracked/sore lips, sore tongue, weakened immune function, skin rashes, seizures, and elevated homocysteine.

Contributors to Deficiency

Vitamin B6 deficiency may be associated with low dietary intake, poor absorption, anorexia, suboptimal vitamin B6 activation, pyroluria, chronic alcoholism, and/or medications such as oral contraceptive pills (OCPs) which deplete vitamin B6, and hydrazines (isoniazid, hydralazine, etc.), sulphydryl compounds (penicillamine, etc.), and substituted hydroxylamines (cycloserine, etc.), which inactivate vitamin B6.

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Increasing vitamin B6 dietary intake[90] or supplementing with the active form of vitamin B6 (pyridoxine-5-phosphate (P5P)), especially if both xanthurenate and kynurenate are elevated, as this more likely is associated with low vitamin B6 levels
- Supporting cofactors to vitamin B6, including lysine, zinc, ATP, and vitamin B2
- Further workup around blood sugar and insulin dysregulation, oxidative stress, and inflammation, as elevated xanthurenate and kynurenate may be associated with an increased type 2 diabetes risk[91], and elevated kynurenate may be associated with oxidative stress and inflammation[92]

See **“Appendix B: Potential Support Considerations” on page 149** for more information.

NOTE

Rule out certain food and supplement intake around the time of testing, as tryptophan may elevate both xanthurenate and kynurenate, and bee products (honey, propolis, etc.), white potatoes, broccoli, basil, and thyme may elevate kynurenate.

PYROGLUTAMATE

Elevated pyroglutamate may be associated with glutathione deficiency. Pyroglutamate is an intermediate in glutathione recycling and production. Glutathione is a complex amino acid produced through the transsulfuration pathway via the conjugation of the amino acids cysteine, glycine, and glutamate. Pyroglutamate is leftover after glutathione is reduced. It can bind to more cysteine and recycle back to glutathione. If the body cannot convert pyroglutamate forward to glutathione, it will show up elevated in the urine.

Glutathione is one of the body’s most potent antioxidants. Glutathione may play an important role in cancer prevention by quenching the downstream reactive quinones of the 4-OH and 2-OH estrogen catechols, that contribute to breast, endometrial, and prostate tissue DNA damage. Glutathione may become depleted with increased inflammation or when glutathione precursors are deficient.

NOTE

Overall, consider assessing for other patterns of high inflammation found on the DUTCH Test, see **“Appendix A: DUTCH Test Patterns” on page 148** *for more information.*

Potential Support Considerations if Elevated

In addition to treating the underlying cause(s) (inflammation, infection, etc.), additional evaluation and support considerations may include:

- Supporting glutathione precursors and cofactors[93]
- Testing genetic SNPs related to glutathione (GPX, GSTP1), and measuring homocysteine and methionine in the serum to ensure adequate levels
- Encouraging healthy estrogen metabolism and clearance to minimize 2-OH and 4-OH conversion to reactive quinones

See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

Rule out Italian cheese (parmesan, etc.) consumption around the time of testing as these cheeses may result in elevated pyroglutamate that is not indicative of a glutathione deficiency.

Potential Support Considerations if Low

Low pyroglutamate may be associated with a need for glutathione support, however, the significance of low pyroglutamate in relation to glutathione levels has not yet been established in the scientific literature. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

b-HYDROXYISOVALERATE

Elevated b-hydroxyisovalerate may be associated with biotin (vitamin B7) deficiency. Biotin is an important cofactor in mitochondrial function, metabolism of fatty acids, glucose, and protein, as well as reactive oxygen species (ROS) production.

Signs & Symptoms

Low biotin may be associated with hair loss, fatigue, tingling and burning in hands and feet, cracked mouth corners, skin rashes, dry skin, dry eyes, depression, nausea, loss of appetite, muscle pain, seizures, and difficulty sleeping.

Contributors to Deficiency

Biotin deficiency may be associated with low dietary intake, poor absorption, dysbiosis and gut health issues, long-term and high dose vitamin B5 supplementation, biotinidase deficiency (a rare inherited disorder), other genetic disorders such as PKU (phenylketonuria), and/or medications such as anti-seizure medications and antibiotics.

Potential Support Considerations if Deficient

In addition to treating the underlying cause(s), additional evaluation and support considerations may include:

- Increasing dietary biotin intake[94]
- Supplementing with biotin
- Ordering a stool test
- Improving gut health

See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

Rule out anti-seizure medications and antibiotics that may be associated with biotin deficiency.

INDICAN

Elevated urinary indican has been shown to be a reliable marker for intestinal dysbiosis. Indican levels do not correlate with the severity of dysbiosis; instead, an elevated indican merely shows that dysbiosis is present.

NOTE

As dysbiosis can sometimes lead to elevations in circulating estradiol levels by interfering with phase 3 detoxification, see **"Phase 3 Detox" on page 114** *for more information. The indican marker may be especially helpful when there is estrogen excess. In fact, it is interesting to note that data from DUTCH samples in postmenopausal females and males have shown a small, but statistically significant positive association between elevated urinary indican levels and estradiol levels.*

Signs & Symptoms

Gut dysbiosis may be associated with bloating, gas, abdominal pain, heartburn, acid reflux, food intolerance, chronic fatigue, skin issues, acne, rashes, mood issues, anxiety, depression, trouble focusing, brain fog, joint aches and pains, inflammation, difficulty with urination, etc.

Contributing Factors

High indican may be associated with protein malabsorption because of low stomach acid, poor pancreatic function, Celiac disease, colon anerobic bacteria overgrowth, small intestinal bacterial overgrowth (SIBO), constipation, and medications that interfere with protein absorption, i.e., PPIs, other antacids, or H2 blockers.

Indican is a byproduct of tryptophan putrefaction by bacteria in the gastrointestinal tract. Therefore, keep in mind that indican may be within range even though dysbiosis is present if the person consumes a low-protein (and low tryptophan) diet. The indican test is not diagnostic but suggests that additional gut health testing may be warranted.

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Supporting gut health and the HPA axis
- Ordering a stool test
- Ensuring adequate daily bowel movements
- Supporting stomach acid production
- Inquiring about medications that can reduce stomach acid, such as PPIs, H2 blockers, and other antacids

See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NEURO-RELATED MARKERS

HOMOVANILLATE (HVA) & VANILMANDELATE (VMA)

Homovanillate (HVA) is the primary dopamine metabolite and vanilmandelate (VMA) is the primary norepinephrine (NE) and epinephrine (EPI) (adrenaline) metabolite. HVA and VMA do not reflect dopamine and NE/EPI levels in the brain, however, they may provide some insight into overall levels in the body.

Dopamine is a brain and adrenal neurotransmitter that is made from tyrosine with BH4 and iron as cofactors. Dopamine can be converted to NE and then onto EPI. The adrenal medulla makes NE and EPI while the adrenal cortex makes cortisol and DHEA. When adrenal hormone output is low, VMA levels may also be low.

NOTE

If symptoms do not match up with the HVA and VMA results, consider prioritizing treatment based on symptoms.

Signs & Symptoms

Low dopamine may be associated with depression, addictions, cravings, apathy, pleasure-seeking behaviors, increased sleepiness, impulsivity, tremors, low motivation, fatigue, and low mood.

High dopamine may be associated with agitation, insomnia, mania, hyperactivity, hyper-focus, high stress, anxiety, and addictions, cravings, and pleasure seeking to maintain high levels.

Low adrenaline may be associated with addictions, cravings, fatigue, low blood pressure, low muscle tone, intolerance to exercise, depression, and decreased alertness.

High adrenaline may be associated with stress, aggression, violence, impatience, anxiety, panic, excess worry and hypervigilance, insomnia, paranoia, increasing tingling and burning, memory loss, pain sensitivity, high blood pressure, and heart palpitations.

Potential Support Considerations if Both HVA & VMA are Low

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Assessing, and if appropriate, treating long-term HPA axis stressors (chronic stress, pain, inflammation, blood sugar dysregulation, etc.) that may be associated with lower cortisol production, and subsequently lower adrenal dopamine, NE, and EPI production
- Supporting dopamine, NE, and EPI precursors and cofactors[95]
- Testing catechol-O-methyltransferase (COMT) and MAO SNPs (as these two enzymes are involved in metabolizing dopamine, NE, and EPI to HVA and VMA)
- Assessing for long-term THC use

See **“Appendix B: Potential Support Considerations” on page 149** for more information.

Potential Support Considerations if Both HVA & VMA are High

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Assessing, and if appropriate, treating short-term HPA axis stressors (stress, strenuous exercise, pain, etc. around time of testing) that may be associated with increased cortisol production, and subsequently higher dopamine, NE, and EPI
- Testing COMT and MAO SNPs
- Supporting COMT (methylation)[96]
- Supporting MAO[97]
- Dopamine, NE, and EPI producing tumors[98]
- Ruling out foods, supplements, and medications that can increase HVA and VMA levels[99]

See **“Appendix B: Potential Support Considerations” on page 149** for more information.

NOTE

Elevated HVA may be associated with Parkinson’s disease.

Potential Support Considerations if HVA is High & VMA is Low

In addition to treating the underlying cause, additional evaluation and support considerations may include assessing for factors that slow dopamine beta-hydroxylase (DBH) enzymatic activity, which converts dopamine to NE. These may include low vitamin C and copper (DBH cofactors), and C. difficile infections. If HVA is above range, then also see suggestions under “If HVA and VMA are high” above. See **“Appendix B: Potential Support Considerations” on page 149** for more information.

Potential Support Considerations if VMA is High & HVA is Low

In addition to treating the underlying cause, additional evaluation and support considerations may include supporting dopamine’s conversion to HVA with B vitamins (vitamin B2, B3, and B6), copper, magnesium, and S-adenosyl-L-methionine (SAMe). Also see above suggestions under “If HVA and VMA are high”. See **“Appendix B: Potential Support Considerations” on page 149** for more information.

QUINOLINATE

Quinolinate is a neurotoxin derived from tryptophan. Elevated quinolinate may be associated with neuroinflammation, but may also be associated with general inflammation, infection, phthalate exposure, low serotonin, low niacin (vitamin B3), and oral tryptophan use.

Signs & Symptoms

Neuroinflammation may be associated with chronic fatigue, difficulty concentrating, slow cognition, brain fog, memory issues, mood issues, anxiety, depression, irritability, anger, chronic pain, headaches, insomnia, etc.

Contributing Factors

Disorders that may be associated with elevated quinolinate include Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron diseases, multiple sclerosis, epilepsy, amyotrophic lateral sclerosis (ALS), and major depressive disorder.

Quinolinate/Kynurenate Ratio

Another pattern of dysfunction may result from an elevation of quinolinate relative to kynurenate, a related metabolite. This is because quinolinate is neurotoxic whereas kynurenate is neuroprotective. Above range quinolinate that is higher relative to kynurenate may imply the presence of neuroinflammation or other causes for increased quinolinate production.

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include:

- Brain support
- Glutathione and other antioxidant support
- Niacin (vitamin B3) supplementation
- Serotonin support[100]
- Identifying and removing sources of phthalate exposure[101] [w]
- An infection workup
- Evaluating for disorders associated with elevated quinolinate, if appropriate

See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

Consider ruling out oral tryptophan consumption within 48 hours of testing, as it can increase quinolinate levels in the urine.

[w] Raley E, et al. Chemical Exposures via Personal Care Products and the Disproportionate Asthma Burden Among the U.S. Black Population. J Allergy Clin Immunol Pract. 2021 Sep;9(9):3290-3292.

6-OH-MELATONIN SULFATE

Low 6-OH-melatonin sulfate may be associated with low overnight melatonin production. Melatonin is a pineal gland hormone that is involved in sleep and wakefulness cycles, reproductive health, energy metabolism, and more. Though most melatonin is produced in the pineal gland, it can also be synthesized in the gut, and to a lesser extent, in the bone marrow, lymphocytes, epithelial cells, and mast cells. Melanocyte stimulating hormone (MSH) controls the production of melatonin. MSH can be inhibited by mold (for example) and low MSH is associated with insomnia and an increased perception of pain.

The DUTCH Test evaluates overnight production and metabolism of melatonin by measuring 6-OH-melatonin sulfate in the waking sample. Even when a person takes a urine sample in the middle of the night, data strongly suggests that the waking sample alone still correlates best with overnight melatonin production.

Signs & Symptoms

Low melatonin may be associated with insomnia, poor immune response, constipation, weight gain, and increased appetite. High melatonin may be associated with drowsiness, headaches, nausea, dizziness, and mood issues. Elevated melatonin may be associated with chronic fatigue syndrome (CFS).

NOTE

When the DUTCH Test melatonin is above range, it is most commonly due to supplemented oral melatonin, and does not necessarily indicate that the dose is too high. It is not uncommon for supplemental melatonin to cause results to be significantly elevated on testing.

Potential Support Considerations if Low

In addition to treating the underlying cause, additional evaluation and support considerations may include improving sleep hygiene, avoidance of blue light before bedtime, and supplementing with melatonin, tryptophan and/or 5-HTP[102]. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Support sulfation, as the melatonin metabolite that DUTCH measures (6-OH-melatonin sulfate), is dependent on sulfation, see **"Appendix A: DUTCH Test Patterns" on page 148** for more information.

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include assessing for gastrointestinal inflammation, ruling out oral melatonin supplementation taken around the time of testing[103], and ruling out melatonin-containing foods consumed around the time of testing that can result in elevated urinary melatonin levels[104]. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

NOTE

Elevated melatonin production that is problematic is rare (for example, due to a pineal tumor). Please note that oral melatonin supplementation will result in significantly increased DUTCH Test melatonin levels.

8-HYDROXY-2-DEOXYGUANOSINE (8-OHDG)

Elevated 8-hydroxy-2-deoxyguanosine (8-OHdG) may be associated with oxidative stress and DNA damage. 8-OHdG is considered pro-mutagenic and is a biomarker for various cancer and degenerative disease initiation and promotion states.

Elevated 8-OHdG may be associated with chronic inflammation, chronic stress, COPD, hypertension, hyperglycemia, diabetes, diabetic neuropathy, kidney and liver disease, inflammatory bowel disorders, chronic skin issues (psoriasis, eczema, etc.), atherosclerosis and cardiovascular mortality in people with an acute coronary syndrome, Parkinson's disease, depression, insomnia, breast, bladder, and prostate cancers, and more. [x] A 2019 meta-analysis documented that a low 8-OHdG was associated with a worse breast cancer prognosis. [y]

NOTE

The DUTCH Test 8-OHdG reference range is narrower than the standard laboratory range because DUTCH uses functional medicine laboratory ranges. Therefore, an elevated 8-OHdG may prompt additional testing especially if results exceed the standard laboratory range of 0-6ng/mg for females and 0-10ng/mg for males.

Signs & Symptoms

Short-term inflammation may be associated with pain, redness, swelling, heat, etc. Long-term inflammation may be associated with fatigue, joint pain, body pain, weight gain, frequent infections, headaches, mood issues, blood sugar dysregulation, sleep issues, gut health issues, acne, etc.

Potential Support Considerations if Elevated

In addition to treating the underlying cause, additional evaluation and support considerations may include increasing antioxidant support with vitamin C, vitamin E, alpha lipoic acid (ALA), NAC, magnesium, taurine, green tea, curcumin, and colorful fruits and vegetables. See **"Appendix B: Potential Support Considerations" on page 149** for more information.

Assess for other patterns of high inflammation found on the DUTCH Test, see **"Appendix A: DUTCH Test Patterns" on page 148** for more information.

[x] Gohbara M, et al. Clinical impact of admission urinary 8-hydroxydeoxyguanosine level for predicting cardiovascular mortality in patients with acute coronary syndrome. Heart Vessels. 2021; 36(1): 38-47.

[y] Qing X, et al. Prognostic significance of 8-hydroxy-2′-deoxyguanosine in solid tumors: a meta-analysis. BMC Cancer. 2019; 19(1): 997.

ANNOTATIONS

86 Good dietary sources of vitamin B12 include grass-fed beef and beef liver, sardines, lamb, wild caught salmon, nutritional yeast, and eggs.

87 Active forms of vitamin B12 include methyl, hydroxy, and adenosyl cobalamin.

88 Contributors to low stomach acid include stress, older age, H. pylori, SIBO, and certain medications such as H2 blockers, proton pump inhibitors (PPIs), and other antacids.

89 SNPs that may affect vitamin B12 levels include fucosyltransferase 2 (FUT2), transcobalamin (TCN), methionine synthase reductase (MTRR), methylmalonyl CoA mutase (MUT), and metabolism of cobalamin associated B (MMAB).

90 Good dietary sources of vitamin B6 include grass-fed beef, turkey breast, pinto beans, avocado, pistachios, chicken, sesame seeds, and sunflower seeds.

91 Xanthurenate can bind with iron and create a complex that causes oxidative damage in the body. This damage can increase the risk for diabetes and blood sugar/insulin issues and may also result in DNA damage and elevated 8-OHdG.

92 Elevated kynurenate may be associated with chronic stress, high cortisol, oxidative damage, inflammation, bacterial lipopolysaccarides (LPS) from gram negative bacteria, estrogen excess, and a ketogenic diet. These conditions can increase the need for vitamin B6. Kynurenate elevations may be associated with schizophrenia, Alzheimer's disease, cluster headaches, chronic migraines, and psychosis. Kynurenate is neuroprotective, whereas quinolinate is neurotoxic. See **"Quinolinate" on page 141** for more information.

93 Glutathione precursors and cofactors include protein, glycine, N-acetylcysteine (NAC), vitamin B2, B6, vitamin C, selenium, and zinc.

94 Good dietary sources of biotin include egg yolks, organ meats, legumes, lentils, peas, sunflower seeds, seafood, mushrooms, cauliflower, carrots, and barley.

95 Dopamine, NE, and EPI production can be supported with adequate dietary protein, tyrosine, B vitamins, vitamin C, healthy iron levels, biopterin, mucuna extract (L-DOPA), and/or D,L-phenylalanine (DLPA). Use caution with mucuna and DLPA when antidepressant medications are being used and avoid DLPA in people with phenylketonuria (PKU).

96 Methylation support includes active B vitamins, magnesium, choline, trimethylglycine (TMG or betaine), zinc, methionine, and SAMe.

97 MAO support includes vitamin B2, vitamin B3, vitamin B6 (active form is P5P), optimizing iron and ferritin, magnesium, and lithium orotate. Lithium orotate contains micro doses of lithium, however, it is prudent to make sure thyroid and kidney function are normal before starting. Also consider checking a baseline electrocardiogram (ECG) before starting lithium orotate.

98 Tumors are rare and a high HVA and/or VMA alone are not diagnostic of a tumor.

99 Foods, supplements, and medications that may be associated with increased HVA and VMA levels include bananas, avocados, fava beans, tyrosine, DLPA, L-Dopa, mucuna, quercetin, Parkinson's medications, SNRI medications (Wellbutrin/Bupropion is the most common), tricyclic antidepressants, amphetamines/amphetamine-like medications or drugs, appetite suppressants, caffeine, ephedrine (including Sudafed), and opioids.

100 Serotonin support may include dietary protein, tryptophan, 5-HTP, and vitamin B6. Use caution with tryptophan and 5-HTP when antidepressant SSRI or SNRI medications are used due to the possibility of serotonin syndrome.

101 Phthalates are in hundreds of products, including some shampoos, hair sprays, soaps, deodorants, nail polish, lotions, plastic packaging, garden hoses, medical tubing, vinyl flooring, lubricating oils, carpet backings, adhesives, etc. Phthalates are often added to plastics to make them soft. They are found at higher levels in Black and Hispanic females. They are often found in "fragrances." Visit the Environmental Working Group's website at www.EWG.org for more information on cleaner products that are phthalate-free.

102 Use caution with tryptophan and 5-HTP when antidepressant SSRI or SNRI medications are being used due to the possibility of serotonin syndrome.

103 Due to the first-pass gut and liver effect, oral melatonin results in very high urinary melatonin metabolite levels that do not reflect circulating melatonin levels and cannot be used to determine if the oral melatonin dose is appropriate. The melatonin test is only useful as a baseline marker when melatonin is not taken the night before collecting.

104 Melatonin-containing foods include tomatoes, walnuts, barley, rye, strawberries, cherries, red wine, beer, pineapples, bananas, oranges, brown rice, pistachios, and corn. These 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.

SECTION EIGHT

References

Table of Contents

Appendices 138

APPENDIX A: DUTCH TEST PATTERNS 138
APPENDIX B: POTENTIAL SUPPORT CONSIDERATIONS 139
APPENDIX C: DUTCH TEST SAMPLE REPORT 143
APPENDIX D: DUTCH TEST REFERENCE RANGES 149

Glossary 153

Bibliography 157

List of Contributors 159

APPENDIX A: DUTCH TEST PATTERNS

High Inflammation

Inflammation is crucial for protecting the body during injury and infection—however, it can be damaging to tissues if it becomes chronic. Inflammation levels in the body may elevate due to injury, infection, stress, insulin resistance, exposure to pollution or chemicals, autoimmune processes, detoxification issues, standard American diet, poor sleep, sedentary lifestyle, overexercising, and more. Short-term inflammation may be associated with pain, redness, swelling, heat, and loss of function. Long-term inflammation may be associated with fatigue, joint pain, body pain, weight gain, frequent infections, headaches, mood issues, blood sugar dysregulation, sleep issues, gut health issues, acne, etc.

High inflammation is associated with the following results observed on the DUTCH Test:

- 5a-Reductase upregulated
- Aromatase upregulated (testosterone and androstenedione to E2 and E1, respectively)
- DHEA-S lower compared to metabolites etiocholanolone and androsterone
- Estrogen clearance favoring 4-OH and/or 16-OH
- Elevated cortisol metabolism rate
- Elevated free cortisol
- Cortisol metabolism favoring THF when acute, and THE when chronic
- Elevated kynurenate
- Low or elevated pyroglutamate
- Elevated indican
- Elevated quinolinate
- Elevated 8-OHdG

NOTE

Total DHEA is a calculated by adding DHEA-S + etiocholanolone + androsterone. When assessing if DHEA-S is low relative to total DHEA, compare the direction of the dials, not the numbers in the dials.

Suboptimal Sulfation:

Sulfur is the third most abundant mineral in our body. Sulfation pathways support the detoxification of estrogen (and other hormones), bodily waste products, pharmaceutical drugs, toxic compounds, free radicals, and reactive oxygen species (ROS). Sulfur amino acids, such as methionine and cysteine are paramount for SAMe production, and glutathione production. [z]

Suboptimal sulfation is associated with the following results observed on the DUTCH Test:

- Low melatonin. Adequate sulfation is needed to metabolize melatonin into the sulfated metabolite that DUTCH measures (6-OH-melatonin sulfate).
- Elevated 16-OH-E1 relative to 4-OH-E1 and 2-OH-E1, and elevated E3 relative to E1 and E2 (compare the direction of the dials, not the numbers within the dials). 16-OH-E1 and E3 are sulfated and glucuronidated in phase 2. Suboptimal sulfation my result in elevated 16-OH-E1 and E3 urinary levels.

[z] Nimni ME, et al. Are we getting enough sulfur in our diet? Nutr Metab (Lond). 2007 Nov 6; 4:24.

APPENDIX B: POTENTIAL SUPPORT CONSIDERATIONS

For more in-depth potential support considerations, see the DUTCH Treatment Guide.

The information in this handout is provided for informational and educational purposes only and is not medical or treatment advice. Any information and statements regarding dietary or herbal supplements have not been evaluated by the Food and Drug Administration and are not intended to diagnose, treat, cure, or prevent any disease. The use of any information provided in this handout is solely at your own risk. There is limited research on these supplements and their clinical impact.

Adaptogenic Herbs

- Ashwagandha (Withania somnifera)
- Bacopa (Bacopa monnieri) Cordyceps (Cordyceps sinensis or militaris)
- Holy basil (Ocimum tenuiflorum)
- Jujube (Ziziphus jujuba)
- Licorice root (Glycyrrhiza globra)
- Maca (Lepidium meyenii or peruvianum)
- Mimosa (Albizia julibrissin or lebbeck)
- Korean Ginseng (Panax ginseng)
- Reishi (Ganoderma lucidum)
- Rhodiola (Rhodiola rosea) Shatavari (Asparagus racemosus)
- Schizandra (Schisandra chinensis)
- Siberian Ginseng (Eleutherococcus senticosus)
- Skullcap (Scutellaria lateriflora)

NOTE

Licorice root limits the deactivation of free cortisol into free cortisone and caution is warranted in individuals with hypertension. Licorice root can worsen hypertension. Always monitor blood pressure and electrolyte balance when using licorice root.

Blood Sugar

Blood sugar balancing substances may include inositol, berberine, chromium, cinnamon, Gymnema, ALA, etc.

Bone Health

Supportive treatments for bone health may include hormone replacement therapy (HRT) if appropriate, resistance training, vitamin D3, vitamin K2Mk7, calcium, magnesium, phosphorus, optimizing thyroid function, and optimizing the HPA axis, etc.

Brain

Brain support may include a nutrient-rich diet, movement to improve blood flow to the brain, stress reduction, vagal nerve stimulation, blood sugar regulation, smoking cessation, anemia treatment, hemochromatosis treatment, sleep apnea treatment, neurofeedback, acupuncture, chiropractic, massage, hyperbaric oxygen treatment (HBOT), craniosacral treatment, rosemary, bacopa monnieri, fish oil, gingko biloba, cordyceps sinesis, maca lepidium, pyrroloquinoline quinone (PQQ), and optimizing sex hormone levels.

Calming Herbs

Calming herbs may include milky oats, ziziphus (jujube), valerian, passionflower, chamomile, lemon balm, California poppy (may also reduce pain), etc.

Estrogen Detox

General estrogen detox support may include DIM/I3C, broccoli sprouts (contain glucoraphanin/sulforaphane), cruciferous vegetables, apiaceae carrot family vegetables, rosemary, turmeric, calcium-d-glucarate, resveratrol, quercetin, glutathione, NAC, fiber, hydration, and adequate bowel movements.

NOTE

> *DIM and I3C induce CYP-1a1 (the preferred pathway), however they also tend to lower E1 and E2, which may not be appropriate for every person.*

GABA

GABA support may include GABA, L-theanine, pregnenolone, Honokiol (extracted from magnolia off. bark), lemon balm, passionflower, valerian, etc.

Glutathione & Antioxidants

Glutathione and antioxidant support may include glutathione, NAC, sulforaphane (highest in broccoli sprouts), green tea, protein, selenium, vitamins E and C, etc.

Herbal Anti-Androgens

Herbal anti-androgens used in females may include spearmint tea, chaste tree, licorice, white peony, green tea, black cohosh, and red reishi.

NOTE

> *Licorice root limits the deactivation of free cortisol into free cortisone and caution is warranted in individuals with hypertension.*
>
> *Licorice root can worsen hypertension. Always monitor blood pressure and electrolyte balance when using licorice.*

Herbal Estrogen

Herbal estrogen support may include maca, black cohosh, dong quai, and ground flaxseeds.

Herbal Support for High Cortisol

High cortisol supportive herbs may include ashwagandha, skullcap, holy basil, cordyceps, jujube, mimosa, chamomile, lemon balm, passionflower, valerian, milky oats, magnolia, and California poppy.

Herbal Support for Low Cortisol

Low cortisol supportive herbs may include ashwagandha, Korean ginseng, rhodiola, cordyceps, schizandra, bacopa, licorice, and panax ginseng.

NOTE

> *Licorice root limits the deactivation of free cortisol into free cortisone and caution is warranted in individuals with hypertension.*
>
> *Licorice root can worsen hypertension. Always monitor blood pressure and electrolyte balance when using licorice.*

Herbal Testosterone

Herbal testosterone support may include tongkat ali, ashwagandha, Mucuna, Tribulus[aa], Epimediummaca, damiana, forskolin, and fenugreek. Shatavari is additionally used in females.

Hypothalamic-Pituitary-Adrenal (HPA) Axis

HPA axis support may include B vitamins, vitamin C, adaptogenic herbs, stress reduction, blood sugar regulation, weight loss if appropriate, etc.

Inflammation

Inflammation reducing substances include NAC, turmeric, resveratrol, alpha lipoic acid (ALA), fish oil, EGCG, mushroom therapies, etc. Inflammation can also be reduced with stress reduction, exercise (in moderation), anti-inflammatory diet, autoimmune protocol (AIP) diet, blood sugar regulation, and by limiting exposure to environmental toxic compounds.

Liver

Liver supporting substances may include B vitamins, amino acids, NAC, choline, ALA, dandelion, artichoke, turmeric, milk thistle, etc.

Mitochondria

Mitochondria supporting substances include B vitamins, NADH, vitamin C, vitamin E, L-carnitine, NAC, ALA, curcumin, resveratrol, CoQ10/ubiquinol, PQQ, sulforaphane, glutathione support, selenium, magnesium, manganese, and copper. Mitochondrial function can also be supported by improving a standard American diet, limiting alcohol intake, reducing environmental chemical exposures, treating chronic and acute disease and infections, intermittent fasting, exercise, and cold exposure (ending showers with 30 seconds of cold water for example).

Natural 5a Blockers

Natural 5a blockers may include saw palmetto and beta-sitosterol, reishi, nettle root, Pygeum africanum, polyunsaturated fats (PUFAs), and epigallocatechin gallate (EGCG).

Natural Aromatase Inducers

Natural aromatase inducers may include forskolin (Indian coleus), quercetin, rutin, white peony and licorice.

NOTE

> *Licorice root limits the deactivation of free cortisol into free cortisone and caution is warranted in individuals with hypertension.*
>
> *Licorice root can worsen hypertension. Always monitor blood pressure and electrolyte balance when using licorice.*

[aa] Munir N, et al. Therapeutic Response of Epimedium gandiflorum's Different Doses to Restore the Antioxidant Potential and Reproductive Hormones in Male Albino Rats. Dose Response. 2020 Sep 14; 18(3)

Natural Aromatase Inhibitors

Natural aromatase inhibitors may include chrysin, damiana, mangosteen, and Agaricus (white button mushroom), active lifestyle, EGCG, flaxseed, and grape seed extract (GSE).

Parasympathetic Nervous System (PNS)

PNS support may include meditation, prayer, breath work, and vagal nerve stimulation (humming, singing loudly, gargling), etc.

Phase 2 Glucuronidation

Glucuronidation support may include sulforaphane and calcium-d-glucarate.

Phase 2 Methylation

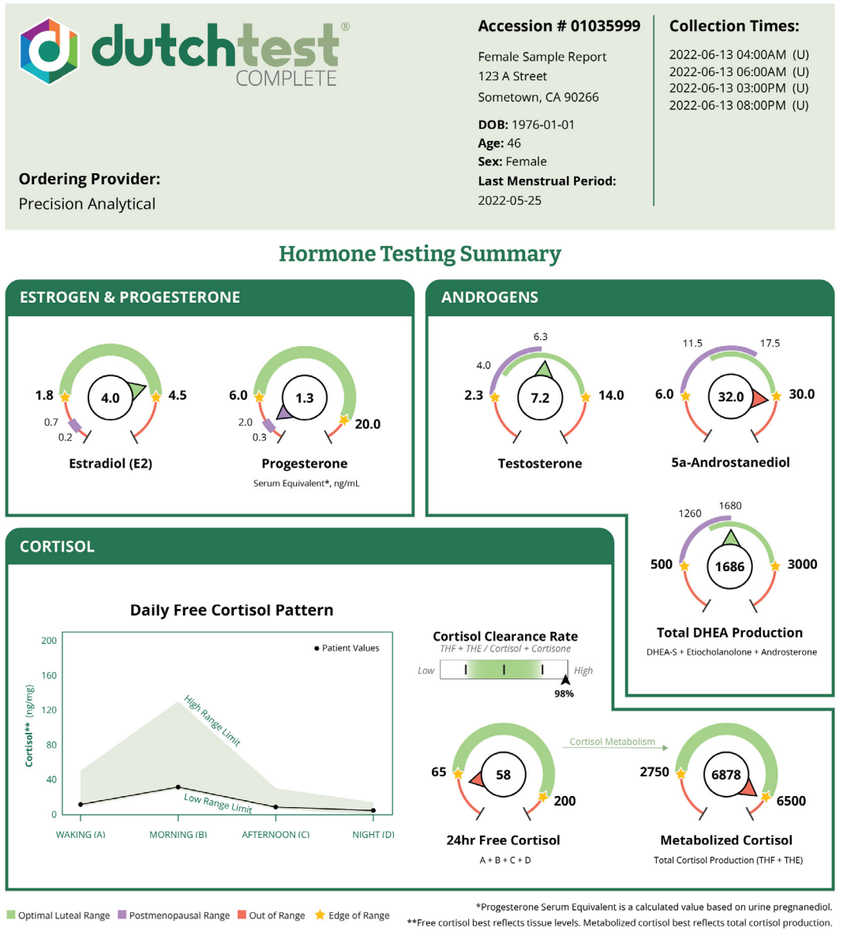
Methylation support may include active B vitamins, magnesium, choline, trimethylglycine (TMG), methionine, zinc, and SAMe.

Phase 2 Sulfation

Sulfation support includes supplementing with the cofactor molybdenum and sulfur donors such as methionine, NAC, methylsulfonylmethane (MSM), taurine, sulforaphane, glutathione, and/or sulfurous vegetables such as onions, garlic, eggs, brassicas, asparagus, arugula, etc. Assess for and lower inflammation, as inflammation is associated with decreased sulfation. Assess for possible hypothyroidism with a full thyroid panel (TSH, fT4, fT3, RT3, TPO and TG antibodies) as hypothyroidism may be associated with poor sulfation.

APPENDIX C: DUTCH TEST SAMPLE REPORT

Page 1: Hormone Testing Summary



dutchtest® COMPLETE

Ordering Provider:
Precision Analytical

Accession # 01035999

Female Sample Report
123 A Street
Sometown, CA 90266

DOB: 1976-01-01
Age: 46
Sex: Female
Last Menstrual Period: 2022-05-25

Collection Times:

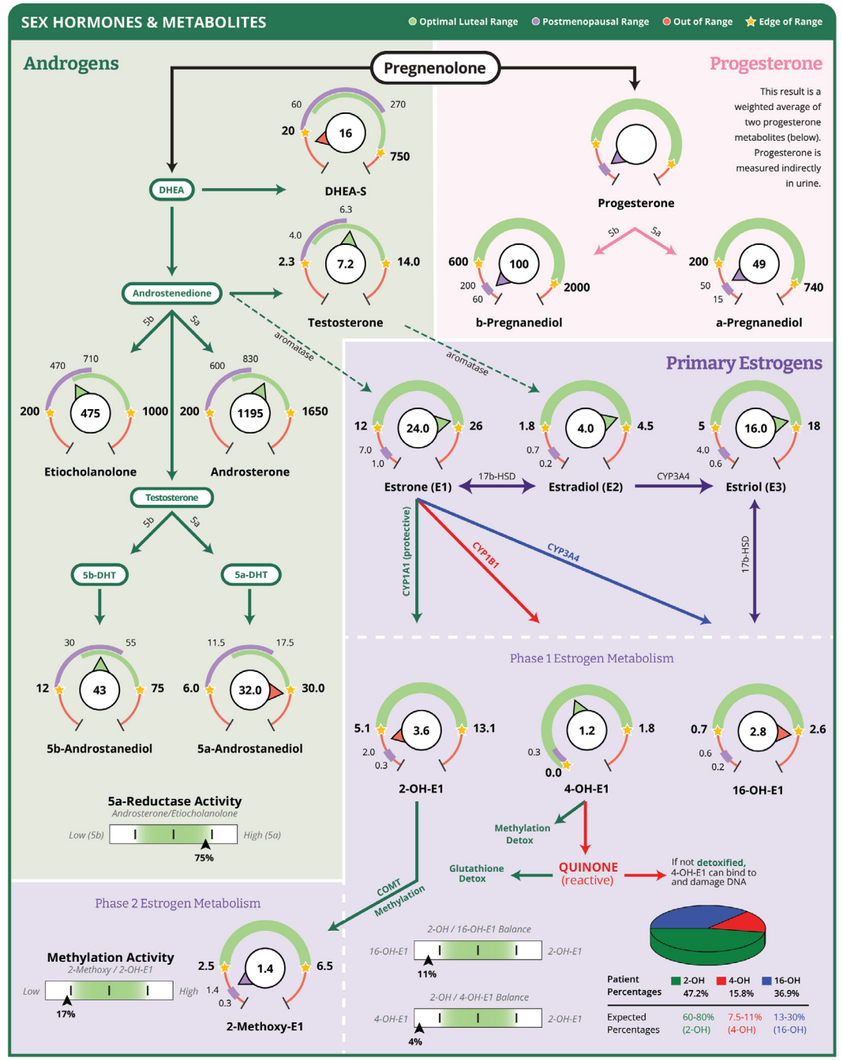
2022-06-13 04:00AM (U)
2022-06-13 06:00AM (U)
2022-06-13 03:00PM (U)
2022-06-13 08:00PM (U)

Sex Hormones & Metabolites

TEST		RESULT	UNITS	LUTEAL*	POSTMENOPAUSAL
Progesterone Metabolites (Urine)					
b-Pregnanediol	Below luteal range	100.0	ng/mg	600 - 2000	60 - 200
a-Pregnanediol	Below luteal range	49.0	ng/mg	200 - 740	15 - 50
Estrogens and Metabolites (Urine)					
Estrone (E1)	High end of luteal range	24.01	ng/mg	12 - 26	1.0 - 7.0
Estradiol (E2)	High end of luteal range	4.00	ng/mg	1.8 - 4.5	0.2 - 0.7
Estriol (E3)	High end of luteal range	16.0	ng/mg	5 - 18	0.6 - 4.0
2-OH-E1	Below luteal range	3.58	ng/mg	5.1 - 13.1	0.3 - 2.0
4-OH-E1	Within luteal range	1.20	ng/mg	0 - 1.8	0 - 0.3
16-OH-E1	Above luteal range	2.80	ng/mg	0.7 - 2.6	0.2 - 0.6
2-Methoxy-E1	Below luteal range	1.35	ng/mg	2.5 - 6.5	0.3 - 1.4
2-OH-E2	Within luteal range	0.74	ng/mg	0 - 3.1	0 - 0.52
4-OH-E2	Within luteal range	0.41	ng/mg	0 - 0.52	0 - 0.12
Total Estrogen	Within range	54.1	ng/mg	35 - 70	3.5 - 15
Metabolite Ratios (Urine)					
2-OH / 16-OH-E1 Balance	Below range	1.28	ratio	2.69 - 11.83	
2-OH / 4-OH-E1 Balance	Below range	2.98	ratio	5.4 - 12.62	
2-Methoxy / 2-OH Balance	Below range	0.38	ratio	0.39 - 0.67	
Androgens and Metabolites (Urine)				**Range**	
DHEA-S	Below range	16.0	ng/mg	20 - 750	
Androsterone	Within range	1195.0	ng/mg	200 - 1650	
Etiocholanolone	Within range	474.6	ng/mg	200 - 1000	
Testosterone	Within range	7.16	ng/mg	2.3 - 14	
5a-DHT	High end of range	6.2	ng/mg	0 - 6.6	
5a-Androstanediol	Above range	32.0	ng/mg	6 - 30	
5b-Androstanediol	Within range	42.6	ng/mg	12 - 75	
Epi-Testosterone	Within range	8.6	ng/mg	2.3 - 14	

* The Luteal Range represents the expected premenopausal luteal range, collected menstrual cycle days 19-22 of a 28-day cycle. If your patient noted taking oral progesterone, the reference range represents the expected range on 100 - 200 mg of oral micronized progesterone (OMP). The ranges in the table below represent ranges in other times of the cycle your patient may have collected, such as follicular or ovulatory phases.

ADDITIONAL NORMAL RANGES	FOLLICULAR	OVULATORY	ON ORAL PG
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



dutchtest® COMPLETE

Ordering Provider:
Precision Analytical

Accession # 01035999

Female Sample Report
123 A Street
Sometown, CA 90266

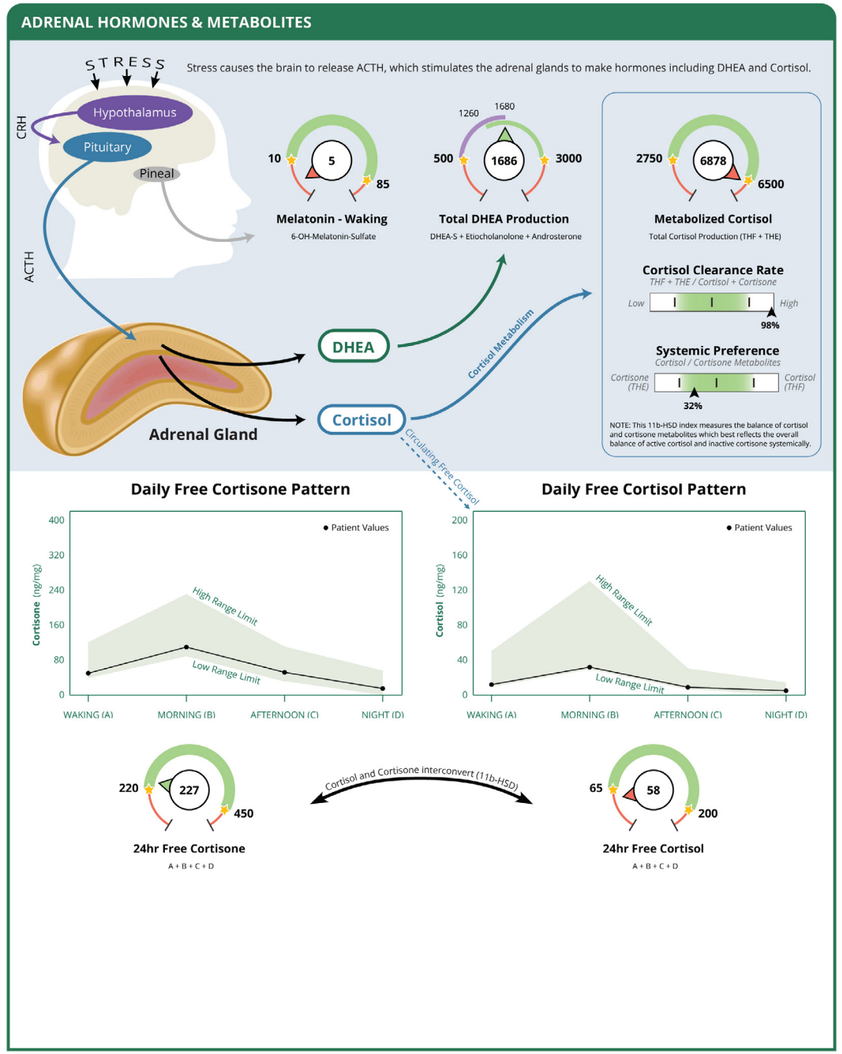
DOB: 1976-01-01
Age: 46
Sex: Female
Last Menstrual Period:
2022-05-25

Collection Times:

2022-06-13 04:00AM (U)
2022-06-13 06:00AM (U)
2022-06-13 03:00PM (U)
2022-06-13 08:00PM (U)

Adrenal Hormones & Metabolites

TEST		RESULT	UNITS	NORMAL RANGE
Daily Free Cortisol and Cortisone (Urine)				
Cortisol A - Waking	Low end of range	12.0	ng/mg	10 - 50
Cortisol B - Morning	Low end of range	32.0	ng/mg	30 - 130
Cortisol C - Afternoon	Low end of range	9.0	ng/mg	7 - 30
Cortisol D - Night	Within range	5.0	ng/mg	0 - 14
Cortisone A - Waking	Low end of range	50.0	ng/mg	40 - 120
Cortisone B - Morning	Low end of range	110.0	ng/mg	90 - 230
Cortisone C - Afternoon	Within range	52.0	ng/mg	32 - 110
Cortisone D - Night	Within range	15.0	ng/mg	0 - 55
24hr Free Cortisol	Below range	58.0	ng/mg	65 - 200
24hr Free Cortisone	Low end of range	227.0	ng/mg	220 - 450
Creatinine (Urine)				
Creatinine A - Waking	Within range	0.50	mg/ml	0.2 - 2
Creatinine B - Morning	Within range	0.72	mg/ml	0.2 - 2
Creatinine C - Afternoon	Within range	0.48	mg/ml	0.2 - 2
Creatinine D - Night	Within range	0.34	mg/ml	0.2 - 2
Cortisol Metabolites and DHEA-S (Urine)				
a-Tetrahydrocortisol (a-THF)	Above range	464.0	ng/mg	75 - 370
b-Tetrahydrocortisol (b-THF)	Within range	2318.9	ng/mg	1050 - 2500
b-Tetrahydrocortisone (b-THE)	Above range	4095.1	ng/mg	1550 - 3800
Metabolized Cortisol (THF + THE)	Above range	6878.0	ng/mg	2750 - 6500
DHEA-S	Below range	16.0	ng/mg	20 - 750
Cortisol Clearance Rate (CCR)	Above range	24.1		6 - 12.5



Page 6: Organic Acid Tests (OATs)

dutchtest® COMPLETE

Accession # 01035999

Female Sample Report
123 A Street
Sometown, CA 90266

DOB: 1976-01-01
Age: 46
Sex: Female
Last Menstrual Period: 2022-05-25

Collection Times:

2022-06-13 04:00AM (U)
2022-06-13 06:00AM (U)
2022-06-13 03:00PM (U)
2022-06-13 08:00PM (U)

Ordering Provider:
Precision Analytical

Organic Acid Tests (OATs)

TEST		RESULT	UNITS	NORMAL RANGE
Nutritional Organic Acids (Urine)				
Vitamin B12 Marker - May be deficient if high				
Methylmalonate (MMA)	Above range	4.9	ug/mg	0 - 2.5
Vitamin B6 Markers - May be deficient if high				
Xanthurenate	Above range	1.23	ug/mg	0.12 - 1.2
Kynurenate	Above range	5.4	ug/mg	0.8 - 4.5
Biotin Marker - May be deficient if high				
b-Hydroxyisovalerate	Within range	7.9	ug/mg	0 - 12.5
Glutathione Marker - May be deficient if low or high				
Pyroglutamate	Within range	42.0	ug/mg	28 - 58
Gut Marker - Potential gut putrefaction or dysbiosis if high				
Indican	Above range	114.0	ug/mg	0 - 100
Neuro-Related Markers (Urine)				
Dopamine Metabolite				
Homovanillate (HVA)	Within range	4.4	ug/mg	3 - 11
Norepinephrine/Epinephrine Metabolite				
Vanilmandelate (VMA)	Within range	4.3	ug/mg	2.2 - 5.5
Neuroinflammation Marker				
Quinolinate	Above range	13.2	ug/mg	0 - 9.6
Additional Markers (Urine)				
Melatonin - Waking				
6-OH-Melatonin-Sulfate	Below range	5.3	ng/mg	10 - 85
Oxidative Stress / DNA Damage				
8-Hydroxy-2-deoxyguanosine (8-OHdG)	Within range	2.6	ng/mg	0 - 5.2

APPENDIX D: DUTCH TEST REFERENCE RANGES

FEMALE REFERENCE RANGES (UPDATED 07.01.2022)					
CATEGORY	BIOMARKER	LOW %	HIGH %	LOW LIMIT	HIGH LIMIT
Sex Hormones: Estrogen	Estrone (E1)	20%	80%	12	26
	Estradiol (E2)	20%	80%	1.8	4.5
	Estriol (E3)	20%	80%	5	18
	2-OH-E1	20%	80%	5.1	13.1
	4-OH-E1	0	80%	0	1.8
	16-OH-E1	20%	80%	0.7	2.6
	2-Me-E1	20%	80%	2.5	6.5
	2-OH-E2	0	80%	0	1.2
	4-OH-E2	0	80%	0	0.5
Sex Hormones: Progesterone	a-Pregnanediol	20%	90%	200	740
	b-Pregnanediol	20%	90%	600	2000
Sex Hormones: Androgen	DHEA-S	20%	90%	20	750
	Androsterone	20%	80%	200	1650
	Etiocholanolone	20%	80%	200	1000
	Testosterone	20%	80%	2.3	14
	5a-DHT	0	80%	0	6.6
	5a-Androstanediol	20%	80%	6	30
	5b-Androstanediol	20%	80%	20	75
	Epi-Testosterone	20%	80%	2.3	14
	a-THF	20%	90%	75	370
	b-THF	20%	90%	1050	2500
	b-THE	20%	90%	1550	3800
Organic Acids: Nutritional	Methylmalonate	0	90%	0	2.2
	Xanthurenate	0	90%	0	1.4
	Kynurenate	0	90%	0	7.3
	b-Hydroxyisovalerate	0	90%	0	12.5
	Pyroglutamate	10%	90%	32	60
	Indican	0	90%	0	100
Organic Acids: Neuro-Related	Homovanillate	10%	95%	4	13
	Vanilmandelate	10%	95%	2.4	6.4
	Quinolinate	0	90%	0	9.6

% = 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."

FEMALE REFERENCE RANGES (UPDATED 07.01.2022)					
CATEGORY	BIOMARKER	LOW %	HIGH %	LOW LIMIT	HIGH LIMIT
Organic Acids: Additional	Melatonin (6-OHMS)	20%	90%	10	85
	8-OHdG	0	90%	0	5.2
Adrenals: Cortisol	Urinary Cortisol (Waking)	20%	90%	10	50
	Urinary Cortisol (Morning)	20%	90%	30	130
	Urinary Cortisol (Afternoon)	20%	90%	7	30
	Urinary Cortisol (Night)	0	90%	0	14
	Salivary Cortisol Waking (W)	20%	90%	1.6	4.6
	Salivary Cortisol (W+30 min.)	20%	90%	3.7	8.2
	Salivary Cortisol (W+60 min.)	20%	90%	2.3	5.3
	Salivary Cortisol (Afternoon)	20%	90%	0.4	1.5
	Salivary Cortisol (Night)	0	95%	0	0.9
	Salivary Cortisol (2-3 am)	0	90%	0	0.9
Adrenals: Cortisone	Urinary Cortisone (waking)	20%	90%	40	120
	Urinary Cortisone (morning)	20%	90%	90	230
	Urinary Cortisone (Afternoon)	20%	90%	32	110
	Urinary Cortisone (Night)	0	90%	0	55
	Salivary Cortisone Waking (W)	20%	90%	6.8	14.5
	Salivary Cortisone (W+30 min.)	20%	90%	12.4	19.4
	Salivary Cortisone (W+60 min.)	20%	90%	9.4	15.3
	Salivary Cortisone Afternoon	20%	90%	2	7.1
	Salivary Cortisone Night	0	95%	0	4.8
	Salivary Cortisone (2-3 am)	0	95%	0	4.8
CALCULATED VALUES					
Androgen	Total DHEA Production	20%	80%	500	3000
Estrogen	Total Estrogens	20%	80%	35	70
Adrenal	Metabolized Cortisol	20%	90%	2750	6500
	Urinary 24hr Free Cortisol	20%	90%	65	200
	Urinary 24hr Free Cortisone	20%	90%	220	450
	Salivary Cortisol Total	20%	90%	9.6	19.3
	Salivary Cortisone Total	20%	90%	36	55

% = 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."

MALE REFERENCE RANGES (UPDATED 07.01.2022)					
CATEGORY	BIOMARKER	LOW %	HIGH %	LOW LIMIT	HIGH LIMIT
Sex Hormones: Androgen	DHEA-S	20%	90%	30	1500
	Androsterone	20%	80%	500	3000
	Etiocholanolone	20%	80%	400	1500
	Testosterone	20%	90%	25	115
	5a-DHT	20%	90%	5	25
	5a-Androstanediol	20%	90%	30	250
	5b-Androstanediol	20%	90%	40	250
	Epi-Testosterone	20%	90%	25	115
	a-THF	20%	90%	175	700
	b-THF	20%	90%	1750	4000
	b-THE	20%	90%	2350	5800
Sex Hormones: Estrogen	Estrone (E1)	10%	90%	4	16
	Estradiol (E2)	10%	90%	0.5	2.2
	Estriol (E3)	10%	90%	2	8
	2-OH-E1	0	90%	0	5.9
	4-OH-E1	0	90%	0	0.8
	16-OH-E1	0	90%	0	1.2
	2-Me-E1	0	90%	0	2.8
	2-OH-E2	0	90%	0	0.6
	4-OH-E2	0	90%	0	0.3
Sex Hormones: Progesterone	a-Pregnanediol	10%	90%	20	130
	b-Pregnanediol	10%	90%	75	400
Organic Acids: Nutritional	Methylmalonate	0	90%	0	3
	Xanthurenate	0	90%	0	2.1
	Kynurenate	0	90%	0	9.3
	b-Hydroxyisovalerate	0	90%	0	18
	Pyroglutamate	10%	90%	43	85
	Indican	0	90%	0	131
Organic Acids: Neuro-Related	Homovanillate	10%	95%	4.8	19
	Vanilmandelate	10%	95%	2.8	8
	Quinolinate	0	90%	0	12.5

% = Population percentile: Example – a high limit of 90% means results higher than 90% of the men tested for the reference range will be designated as "high."

MALE REFERENCE RANGES (UPDATED 07.01.2022)					
CATEGORY	BIOMARKER	LOW %	HIGH %	LOW LIMIT	HIGH LIMIT
Organic Acids: Additional	Melatonin (6-OHMS)	20%	90%	10	85
	8-OHdG	0	90%	0	8.8
Adrenals: Cortisol	Urinary Cortisol (Waking)	20%	90%	13	80
	Urinary Cortisol (Morning)	20%	90%	35	180
	Urinary Cortisol (Afternoon)	20%	90%	10	45
	Urinary Cortisol (Night)	0	90%	0	20
	Salivary Cortisol Waking (W)	20%	90%	1.6	4.6
	Salivary Cortisol (W+30 min.)	20%	90%	3.7	8.2
	Salivary Cortisol (W+60 min.)	20%	90%	2.3	5.3
	Salivary Cortisol (Afternoon)	20%	90%	0.4	1.5
	Salivary Cortisol (Night)	0	95%	0	0.9
	Salivary Cortisol (2-3 am)	0	90%	0	0.9
Adrenals: Cortisone	Urinary Cortisone (waking)	20%	90%	40	160
	Urinary Cortisone (morning)	20%	90%	80	240
	Urinary Cortisone (Afternoon)	20%	90%	40	130
	Urinary Cortisone (Night)	0	90%	0	70
	Salivary Cortisone Waking (W)	20%	90%	6.8	14.5
	Salivary Cortisone (W+30 min.)	20%	90%	12.4	19.4
	Salivary Cortisone (W+60 min.)	20%	90%	9.4	15.3
	Salivary Cortisone Afternoon	20%	90%	2	7.1
	Salivary Cortisone Night	0	95%	0	4.8
	Salivary Cortisone (2-3 am)	0	95%	0	4.8
CALCULATED VALUES					
Androgen	Total DHEA Production	20%	80%	1000	5500
Estrogen	Total Estrogens	10%	90%	10	34
Adrenal	Metabolized Cortisol	20%	90%	4550	10000
	Urinary 24hr Free Cortisol	20%	90%	75	300
	Urinary 24hr Free Cortisone	20%	90%	220	550
	Salivary Cortisol Total	20%	90%	9.6	19.3
	Salivary Cortisone Total	20%	90%	36	55

% = Population percentile: Example – a high limit of 90% means results higher than 90% of the men tested for the reference range will be designated as "high."

16-Hydroxy-Estrone (16-OH-E1)

Phase 1 estrone (E1) metabolite; proliferative estrogen that may contribute to breast tenderness and heavy bleeding, etc.

2-Hydroxy-Estrone (2-OH-E1)

Phase 1 estrone (E1) metabolite; 2-OH metabolites are the most stable phase 1 metabolites.

2-Hydroxy-Estradiol (2-OH-E2)

Phase 1 estradiol (E2) metabolite; 2-OH metabolites are the most stable phase 1 metabolites.

2-Methoxy-Estrone (2-Me-E1)

Phase 2 metabolite that is much more stable than its precursor 2-OH-E1.

4-Hydroxy-Estrone (4-OH-E1)

Phase 1 estrone (E1) metabolite; 4-OH metabolites are the most carcinogenic phase 1 metabolites.

4-Hydroxy-Estradiol (4-OH-E2)

Phase 1 estradiol (E2) metabolite; 4-OH metabolites are the most carcinogenic phase 1 metabolites.

5a-Androstanediol

The best marker of 5a-DHT formation and activity in the tissues; can be used to estimate androgen activity in the tissues.

5a-DHT

Alpha metabolite of testosterone and the most potent androgen; roughly three to four times more androgenic than testosterone.

5b-Androstanediol

Weak beta androgen metabolite of testosterone that does not contribute to symptoms of androgen excess.

8-Hydroxy-2-Deoxyguanosine (8-OHdG)

Marker associated with oxidative stress and DNA damage.

a-Pregnanediol

Progesterone metabolite that modulates gamma-aminobutyric acid (GABA) receptors in the central nervous system (CNS) and can lead to improvements in mood and sleep.

a-Tetrahydrocortisol (a-THF)

Alpha cortisol metabolite.

Androsterone

Alpha DHEA metabolite that is roughly seven times weaker than testosterone; can be used to estimate androgen activity in the tissues.

b-Hydroxyisovalerate

Marker associated with biotin deficiency.

b-Pregnanediol

Most prominent progesterone metabolite, however, doesn't modulate GABA receptors.

b-Tetrahydrocortisol (b-THF)

Beta cortisol metabolite.

b-Tetrahydrocortisone (b-THE)

Beta cortisone metabolite.

Cortisol Awakening Response (CAR)

An HPA axis resiliency marker, an anticipatory marker of upcoming daily events, and a surrogate marker for HPA axis plasticity/reactivity.

Creatinine

A compound that is produced from the metabolism of creatine from the kidneys and excreted in the urine. In order to provide the most accurate results, the DUTCH Test uses creatinine excretion to control for hydration status.

DHEA-S

Sulfated and circulating form of DHEA.

Epi-Testosterone

Not an androgenic hormone but produced in approximately the same amount as testosterone by the testes. Can be used to distinguish a truly low testosterone from a falsely low measurement due to the UGT genetic variant, see **"UGT Deletion" on page 31** for more information.

Estradiol (E2)

Most potent, biologically active estrogen.

Estriol (E3) / 16-Hydroxy-Estradiol (16-OH-E2)

Least potent, weak estrogen derived from estradiol and 16-OH-E1.

Estrone (E1)

Moderate strength estrogen.

Etiocholanolone

Weak beta DHEA metabolite.

Free Cortisol Diurnal Pattern

Daily diurnal (circadian) rhythm of active cortisol that is normally highest in the morning and lowest at night.

Free Cortisone Diurnal Pattern

Daily diurnal (circadian) rhythm of inactive cortisone that is normally highest in the morning and lowest at night. The free cortisone diurnal pattern is used in combination with the free cortisol diurnal pattern to evaluate active free cortisol levels throughout the day, see **"The Cortisone Shadow" on page 122** for more information.

Homovanillate (HVA)

Primary dopamine metabolite.

Indican

Metabolic byproduct of tryptophan metabolism in the gut that elevates with intestinal dysbiosis.

Kynurenate

Metabolic byproduct of tryptophan metabolism that elevates with vitamin B6 deficiency and inflammation.

Melatonin (6-OH-Melatonin Sulfate)

Melatonin metabolite.

Metabolized Cortisol (THE + THF)

Sum of all cortisol and cortisone metabolites.

Methylmalonate (MMA)

Metabolic byproduct of the citric acid cycle that elevates with vitamin B12 deficiency.

Optional Extra Cortisol Sample (Insomnia Sample)

A measurement of cortisol upon waking at night.

Pyroglutamate

An intermediate in glutathione recycling and production that elevates with glutathione deficiency.

Quinolinate

Metabolic byproduct of tryptophan metabolism and neurotoxin that elevates with neuroinflammation and general inflammation.

Testosterone

The major circulating androgen.

Total Estrogen

Represents the sum of all estrogen markers.

Vanilmandelate (VMA)

Primary norepinephrine and epinephrine metabolite.

Xanthurenate

Metabolic byproduct of tryptophan metabolism that elevates with vitamin B6 deficiency.

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Thank You!

We know every sample received by our lab comes from a real person, with a real story.

We are incredibly thankful for the opportunity to serve healthcare practitioners and their patients around the world, and we love hearing stories about how the DUTCH Test profoundly changes lives. This is why we do what we do!

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Scan the QR code or visit dutchtest.com/dutch-testimonial/ to tell us about how DUTCH has helped you get to the root cause and profoundly change lives, one life at a time.

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