Hormone and Urinary Metabolites Assessment Profile

RESOURCE GUIDE

DOCTOR'S DATA INC.

SCIENCE + INSIGHT

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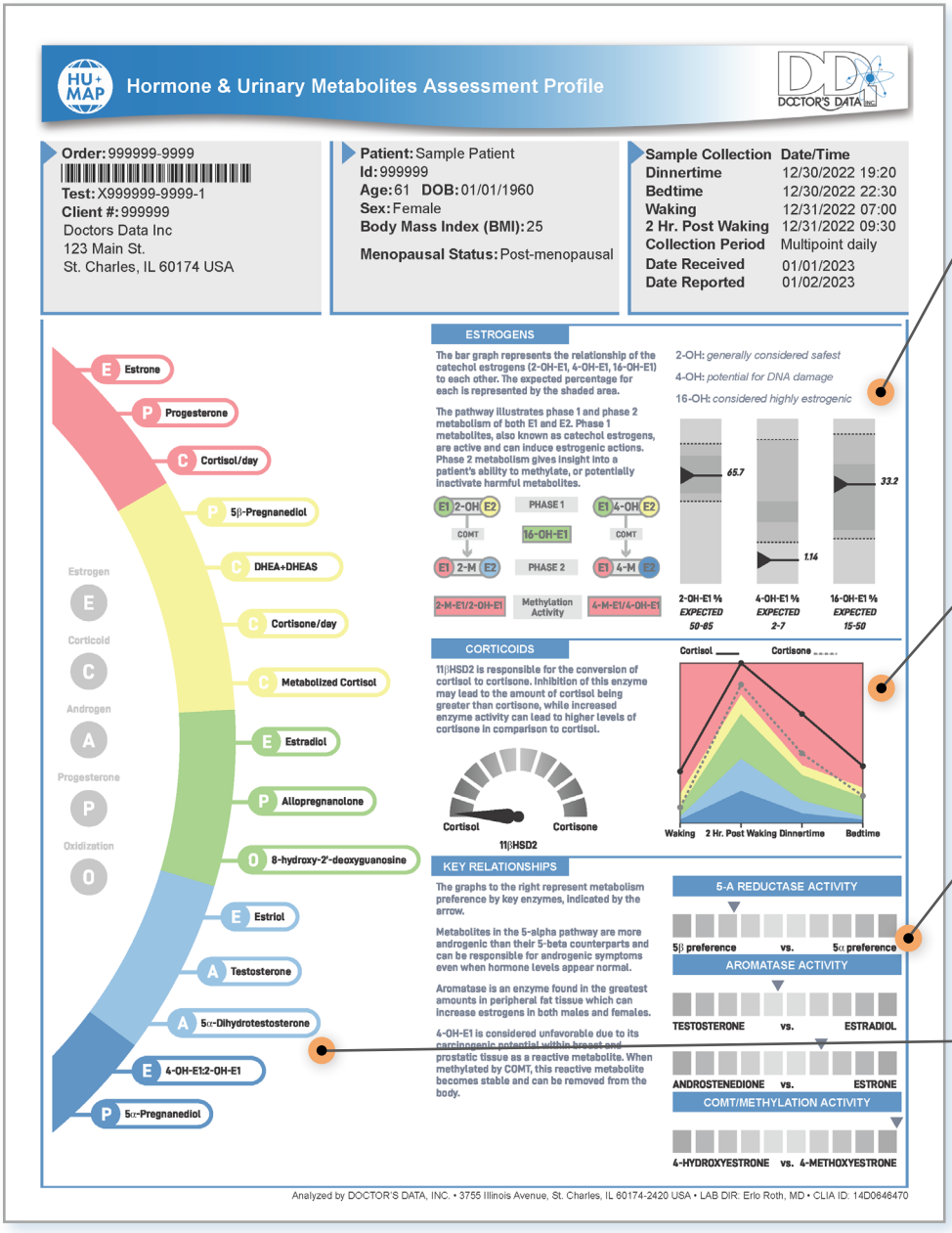
What do the colors on the pathways mean?

The colored analytes allow the clinician to see their patient's results along the metabolic cascade, allowing a quick assessment of potential areas of concern in hormone production and metabolism. Blue indicates low levels of metabolites, green indicates levels are within the reference interval, and red indicates a hormone elevation. Light blue indicates a level is suboptimal, while yellow indicates a level is upper range.



Comparing Phase I vs Phase II estrogen metabolism, methylation activity, and percentage of 2-OH, 4-OH, and 16-OH

Cortisol and cortisone are plotted on the same graph to quickly identify their relationship to each other.

Important relationships are highlighted here, representing metabolism preference by key enzymes.

Important, clinically relevant markers are color-coded to respond to an individual's imbalances, providing an at-a-glance format on the summary page.

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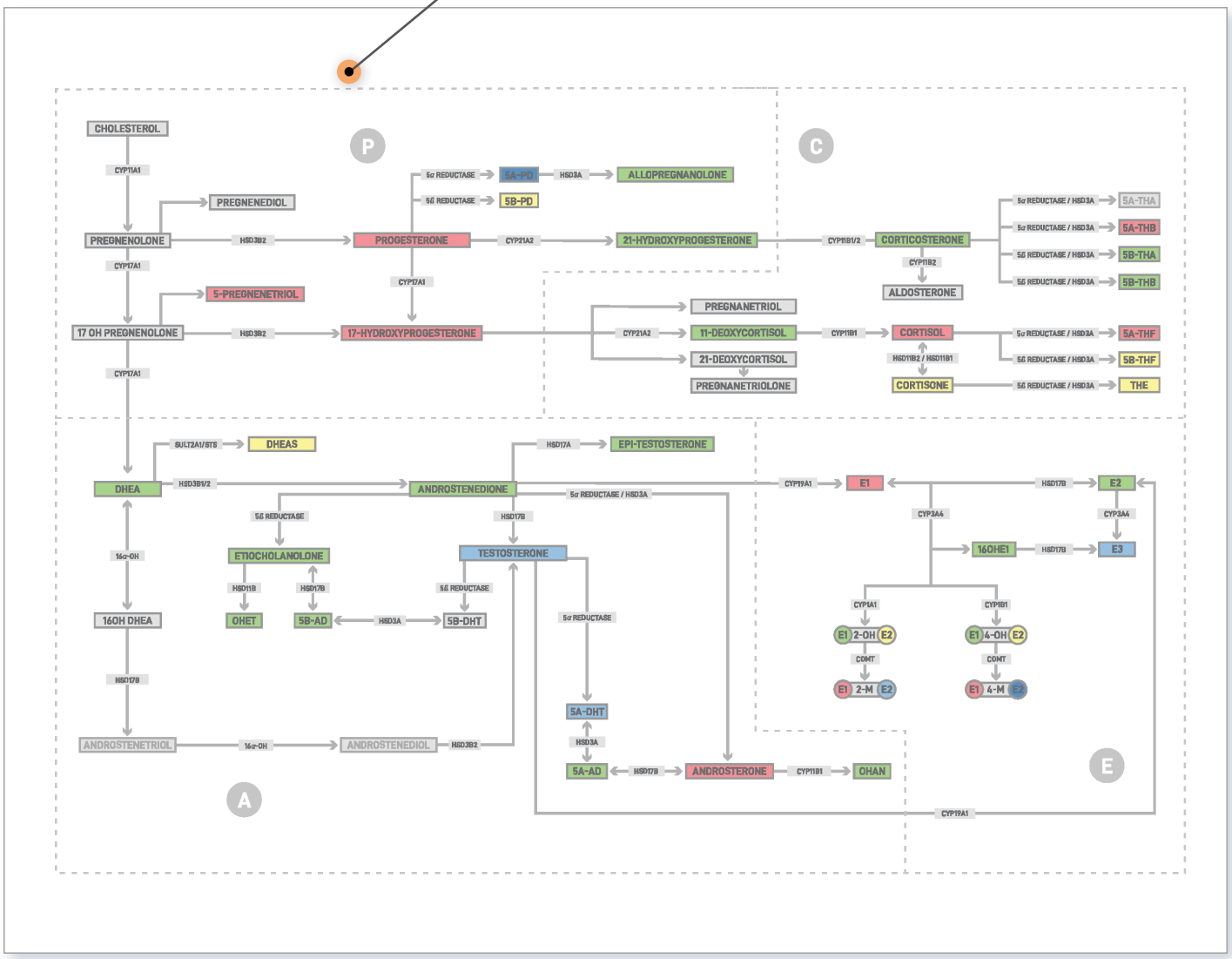
Dynamic color-coded overview of the entire hormone steroid cascade displaying actual results for your patient. Colors are used to easily identify highs and lows for each metabolite.



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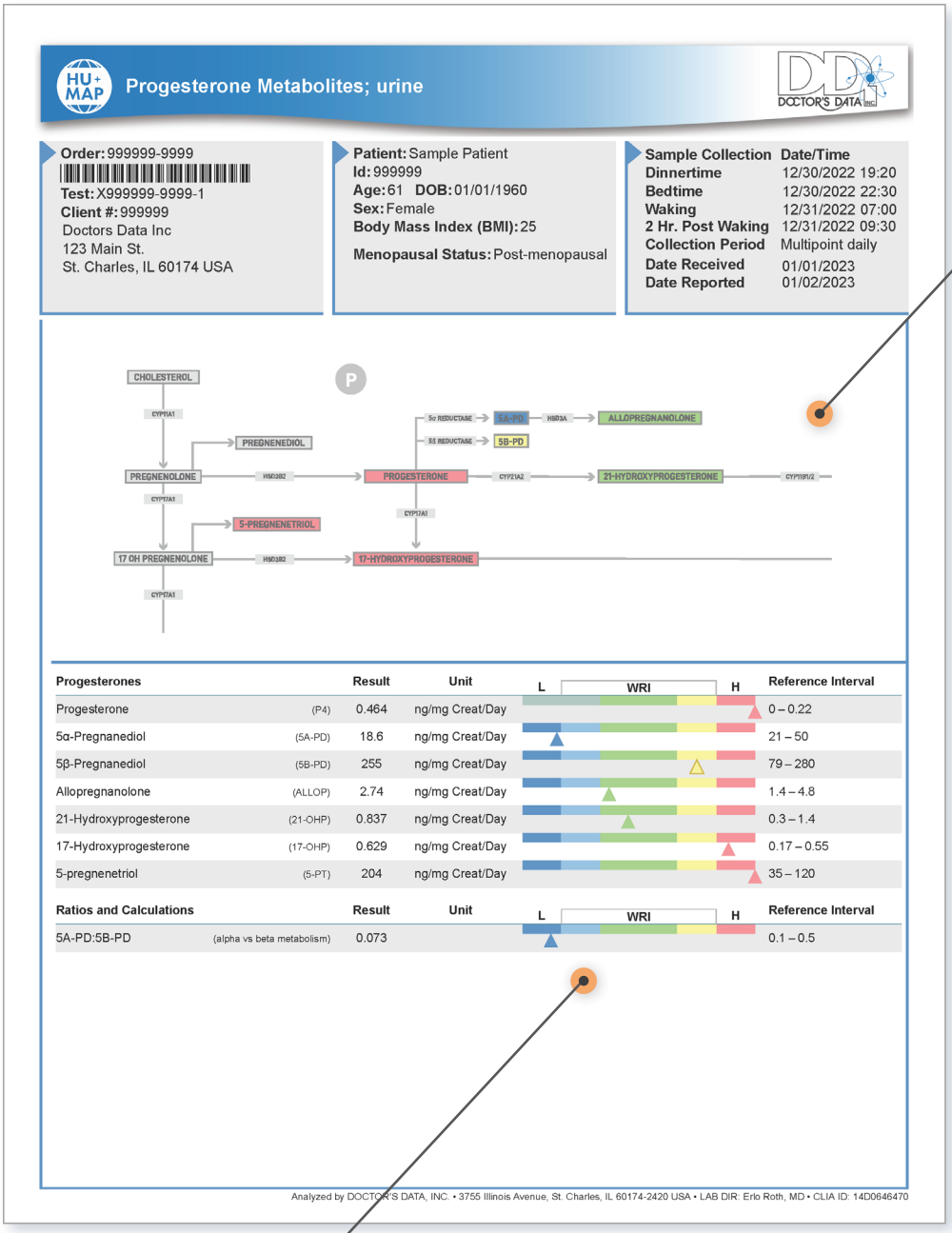

HU+MAP Progesterone Metabolites; urine

DOCTOR'S DATA INC

Order: 999999-9999
Test: X999999-9999-1
Client #: 999999
Doctors Data Inc
123 Main St.
St. Charles, IL 60174 USA

Patient: Sample Patient
Id: 999999
Age: 61 **DOB:** 01/01/1960
Sex: Female
Body Mass Index (BMI): 25
Menopausal Status: Post-menopausal

Sample Collection	Date/Time
Dinnertime	12/30/2022 19:20
Bedtime	12/30/2022 22:30
Waking	12/31/2022 07:00
2 Hr. Post Waking	12/31/2022 09:30
Collection Period	Multipoint daily
Date Received	01/01/2023
Date Reported	01/02/2023

Progesterones		Result	Unit	L WRI H	Reference Interval
Progesterone	(P4)	0.464	ng/mg Creat/Day		0 – 0.22
5α-Pregnanediol	(5A-PD)	18.6	ng/mg Creat/Day		21 – 50
5β-Pregnanediol	(5B-PD)	255	ng/mg Creat/Day		79 – 280
Allopregnanolone	(ALLOP)	2.74	ng/mg Creat/Day		1.4 – 4.8
21-Hydroxyprogesterone	(21-OHP)	0.837	ng/mg Creat/Day		0.3 – 1.4
17-Hydroxyprogesterone	(17-OHP)	0.629	ng/mg Creat/Day		0.17 – 0.55
5-pregnenetriol	(5-PT)	204	ng/mg Creat/Day		35 – 120

Ratios and Calculations		Result	Unit	L WRI H	Reference Interval
5A-PD:5B-PD	(alpha vs beta metabolism)	0.073			0.1 – 0.5

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Progesterone is found in the urine in very small quantities due to its non-polar molecular structure. The HuMap™ not only measures the metabolites of progesterone, but due to our sensitive testing methodologies, also progesterone itself.

Progesterone is often metabolized further down the pathway to allopregnanolone, a metabolite known for its GABA-like effects for sleep and relief of anxiety. The ability to directly test allopregnanolone in the HuMap™ may be of particular interest to practitioners prescribing oral progesterone.

Additionally, 17 hydroxyprogesterone and 21 hydroxyprogesterone can also provide insight into endogenous cortisol and corticosterone production.

Patient results are color-coded to represent highs and lows, as well as values that are within the reference interval, but are trending low (light blue) or trending high (yellow).

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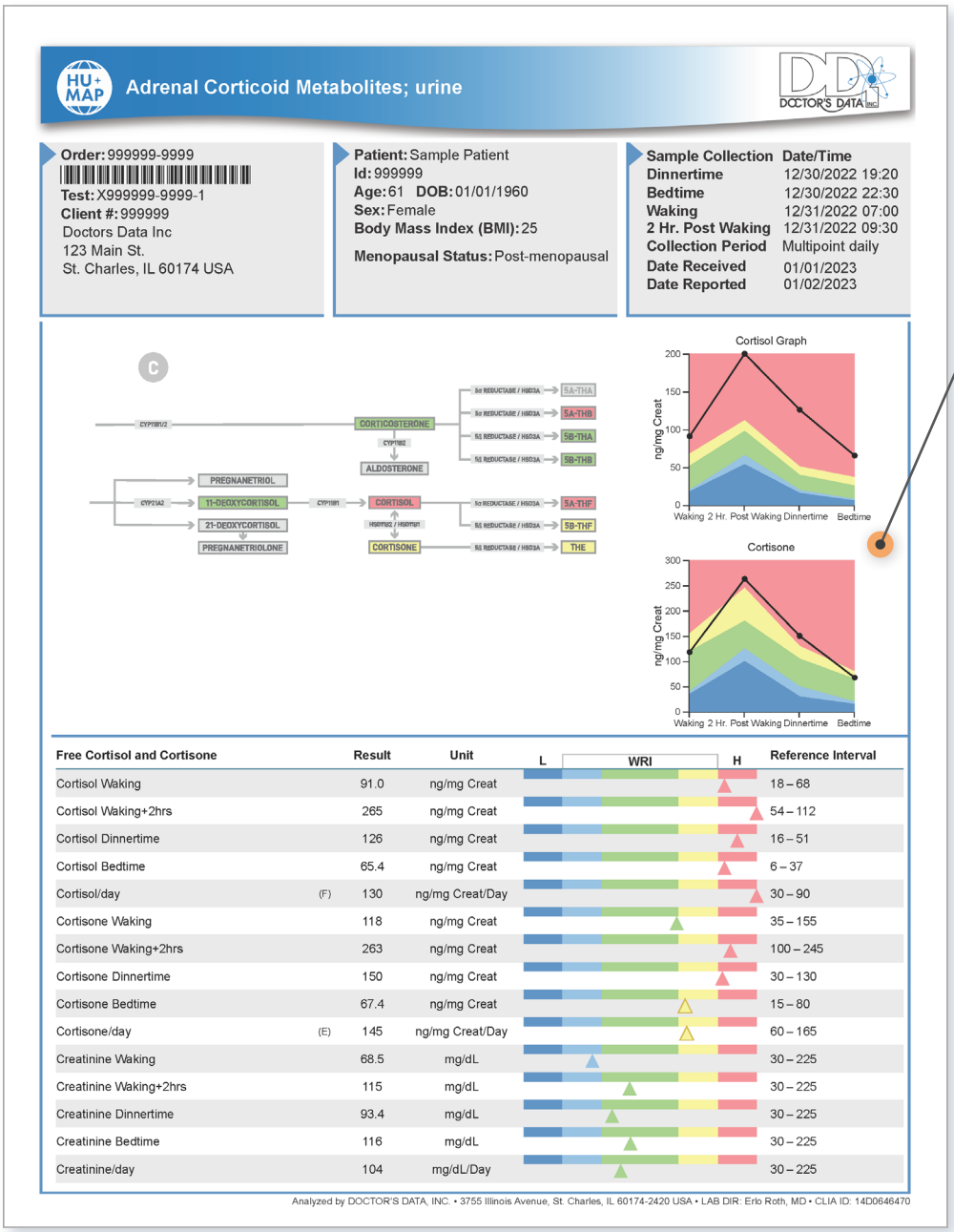

Adrenal Corticoid Metabolites; urine

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Client #: 999999
Doctors Data Inc
123 Main St.
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Collection Period	Multipoint daily
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Free Cortisol and Cortisone		Result	Unit	L WRI H	Reference Interval
Cortisol Waking		91.0	ng/mg Creat		18 – 68
Cortisol Waking+2hrs		265	ng/mg Creat		54 – 112
Cortisol Dinnertime		126	ng/mg Creat		16 – 51
Cortisol Bedtime		65.4	ng/mg Creat		6 – 37
Cortisol/day	(F)	130	ng/mg Creat/Day		30 – 90
Cortisone Waking		118	ng/mg Creat		35 – 155
Cortisone Waking+2hrs		263	ng/mg Creat		100 – 245
Cortisone Dinnertime		150	ng/mg Creat		30 – 130
Cortisone Bedtime		67.4	ng/mg Creat		15 – 80
Cortisone/day	(E)	145	ng/mg Creat/Day		60 – 165
Creatinine Waking		68.5	mg/dL		30 – 225
Creatinine Waking+2hrs		115	mg/dL		30 – 225
Creatinine Dinnertime		93.4	mg/dL		30 – 225
Creatinine Bedtime		116	mg/dL		30 – 225
Creatinine/day		104	mg/dL/Day		30 – 225

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The daily cortisol and cortisone output is graphed in a diurnal pattern displaying the influences of these analytes over the course of the day. The cortisol / cortisone ratio reflects the activity of 11BHSD2 and aids in the understanding of its influence on both cortisol (active) and cortisone (inactive).

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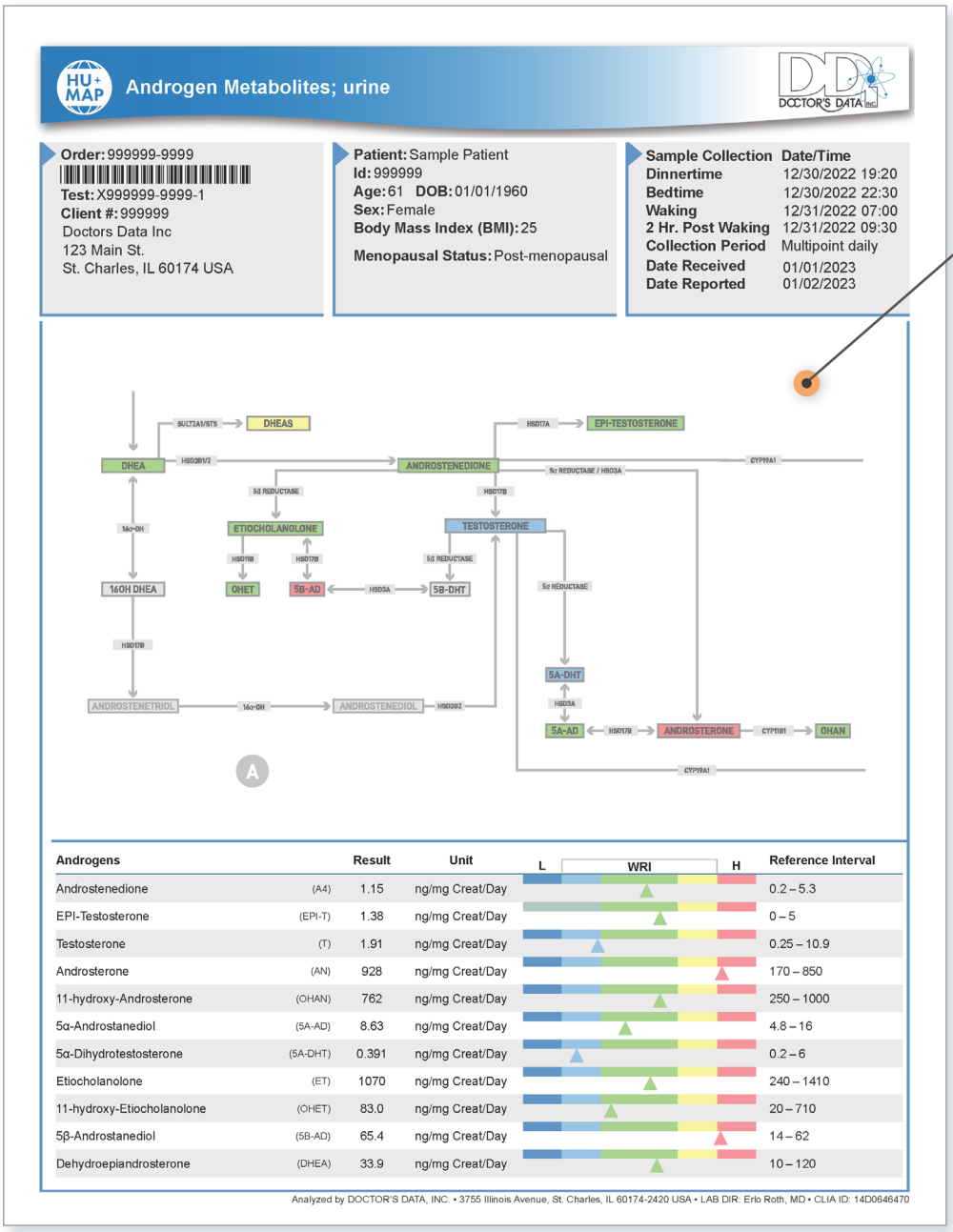

Androgen Metabolites; urine

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Androgens		Result	Unit	L WRI H	Reference Interval
Androstenedione	(A4)	1.15	ng/mg Creat/Day		0.2 – 5.3
EPI-Testosterone	(EPI-T)	1.38	ng/mg Creat/Day		0 – 5
Testosterone	(T)	1.91	ng/mg Creat/Day		0.25 – 10.9
Androsterone	(AN)	928	ng/mg Creat/Day		170 – 850
11-hydroxy-Androsterone	(OHAN)	762	ng/mg Creat/Day		250 – 1000
5α-Androstanediol	(5A-AD)	8.63	ng/mg Creat/Day		4.8 – 16
5α-Dihydrotestosterone	(5A-DHT)	0.391	ng/mg Creat/Day		0.2 – 6
Etiocholanolone	(ET)	1070	ng/mg Creat/Day		240 – 1410
11-hydroxy-Etiocholanolone	(OHET)	83.0	ng/mg Creat/Day		20 – 710
5β-Androstanediol	(5B-AD)	65.4	ng/mg Creat/Day		14 – 62
Dehydroepiandrosterone	(DHEA)	33.9	ng/mg Creat/Day		10 – 120

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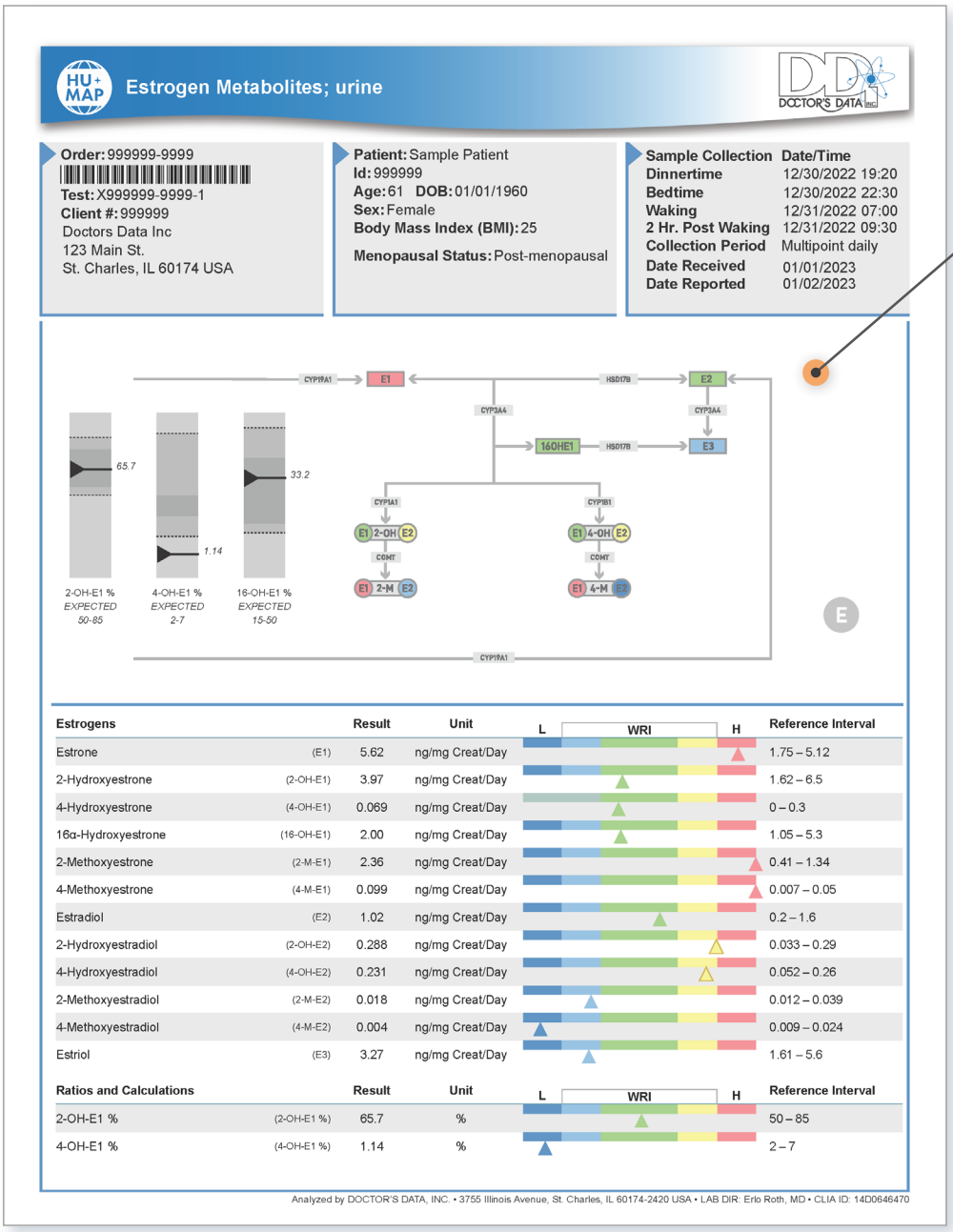
Androgen markers are important to males and females alike. Monitoring the metabolites associated with 5-alpha and 5-beta activity is of particular interest as 5-alpha metabolites are more androgenic. Symptoms associated with higher androgen levels are often seen when levels of 5-alpha reductase and its corresponding metabolites are elevated. 5-beta reductase and its corresponding metabolites are much less androgenic.



HU+MAP Estrogen Metabolites; urine

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Estrogens		Result	Unit	L WRI H	Reference Interval
Estrone	(E1)	5.62	ng/mg Creat/Day		1.75 – 5.12
2-Hydroxyestrone	(2-OH-E1)	3.97	ng/mg Creat/Day		1.62 – 6.5
4-Hydroxyestrone	(4-OH-E1)	0.069	ng/mg Creat/Day		0 – 0.3
16α-Hydroxyestrone	(16-OH-E1)	2.00	ng/mg Creat/Day		1.05 – 5.3
2-Methoxyestrone	(2-M-E1)	2.36	ng/mg Creat/Day		0.41 – 1.34
4-Methoxyestrone	(4-M-E1)	0.099	ng/mg Creat/Day		0.007 – 0.05
Estradiol	(E2)	1.02	ng/mg Creat/Day		0.2 – 1.6
2-Hydroxyestradiol	(2-OH-E2)	0.288	ng/mg Creat/Day		0.033 – 0.29
4-Hydroxyestradiol	(4-OH-E2)	0.231	ng/mg Creat/Day		0.052 – 0.26
2-Methoxyestradiol	(2-M-E2)	0.018	ng/mg Creat/Day		0.012 – 0.039
4-Methoxyestradiol	(4-M-E2)	0.004	ng/mg Creat/Day		0.009 – 0.024
Estriol	(E3)	3.27	ng/mg Creat/Day		1.61 – 5.6

Ratios and Calculations		Result	Unit	L WRI H	Reference Interval
2-OH-E1 %	(2-OH-E1 %)	65.7	%		50 – 85
4-OH-E1 %	(4-OH-E1 %)	1.14	%		2 – 7

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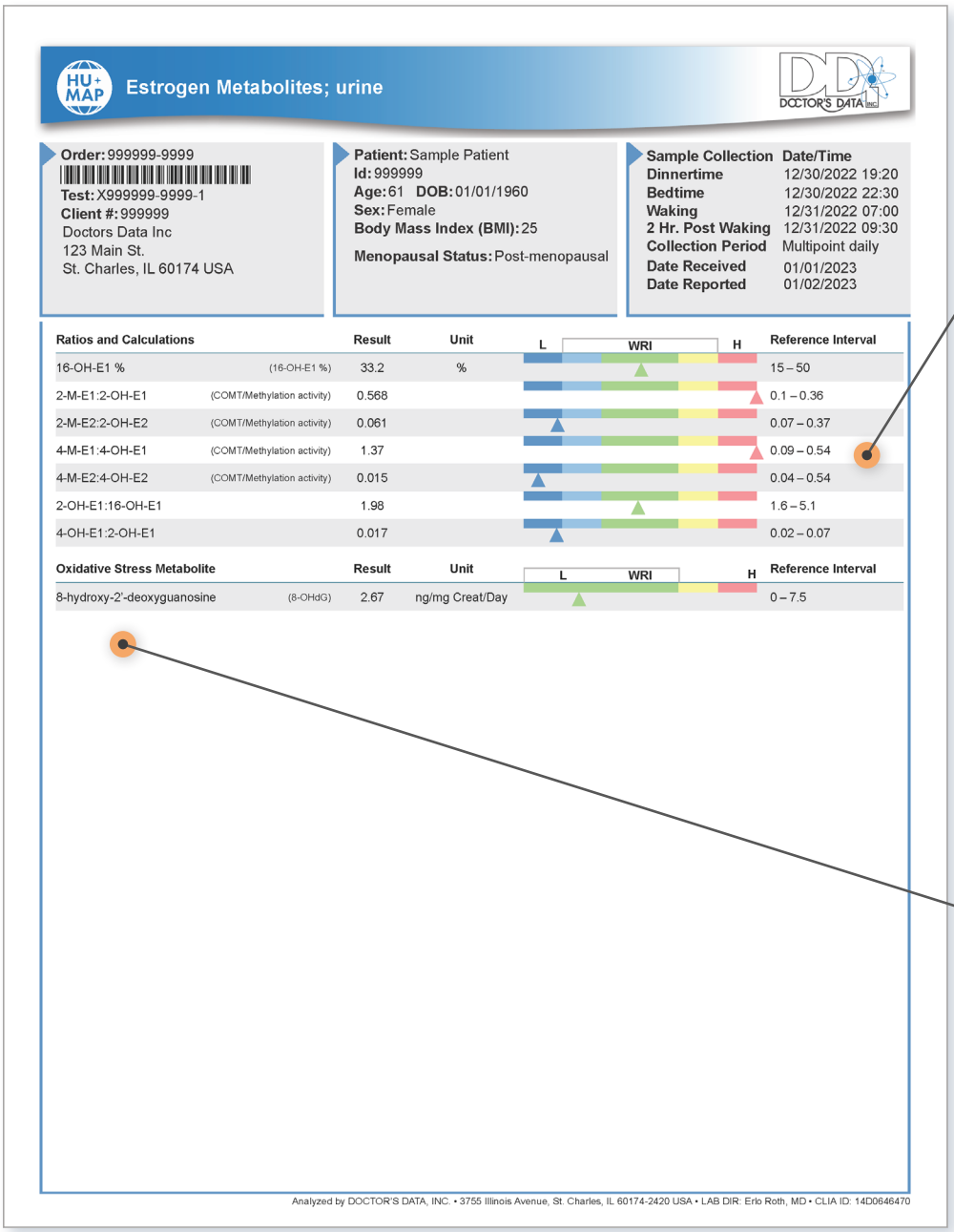
Evaluation of the estrogen metabolism pathway relies on understanding several key steps of metabolism: the amount of unconjugated estrogens, hydroxylation of E1 and E2, methylation of hydroxy estrogens, and the function of key enzymes.



HU+MAP Estrogen Metabolites; urine

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Ratios and Calculations		Result	Unit	L WRI H	Reference Interval
16-OH-E1 %	(16-OH-E1 %)	33.2	%		15 – 50
2-M-E1:2-OH-E1	(COMT/Methylation activity)	0.568			0.1 – 0.36
2-M-E2:2-OH-E2	(COMT/Methylation activity)	0.061			0.07 – 0.37
4-M-E1:4-OH-E1	(COMT/Methylation activity)	1.37			0.09 – 0.54
4-M-E2:4-OH-E2	(COMT/Methylation activity)	0.015			0.04 – 0.54
2-OH-E1:16-OH-E1		1.98			1.6 – 5.1
4-OH-E1:2-OH-E1		0.017			0.02 – 0.07

Oxidative Stress Metabolite		Result	Unit	L WRI H	Reference Interval
8-hydroxy-2'-deoxyguanosine	(8-OHdG)	2.67	ng/mg Creat/Day		0 – 7.5

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The ratio of 4-M E1/E2 to 4-OH E1 / 2 and 2-M E1/E2 to 2-OH E1/E2 can help determine if adequate methylation of catechol estrogens is occurring. The higher the ratio, the higher the likelihood of metabolizing toward the pathway of methylation with lower harm potential, and therefore less reactive quinone formation. Even if 4-OH metabolites are elevated, adequate methylation can indicate these metabolites are being detoxified, rendering them potentially less harmful.

Oxidative stress marker to evaluate potential DNA damage

FAQs

Click here for patient collection FAQs.

What is LC/MS/MS?

Liquid chromatography (LC) tandem mass spectrometry (MS) is an extremely sensitive and specific way to measure many substances. In the case of urinary steroid hormone testing, LC-MS is used to determine both free hormone and their metabolite amounts in urine. Put simply, both parts work together to determine the exact amount of each hormone component in a patient sample. LC separates hormones in a liquid urine sample, which are then injected at various times for MS analysis. MS technology monitors the injected sample for specific hormones based on their molecular weights and expected injection times (commonly called retention times). Tandem MS verifies each hormone identity based on fragmentation and determines its amount. The combination of LC and tandem MS allows for extremely sensitive and specific hormone measurements, even in samples containing similar substances that would interfere with other methods.

What are the advantages of liquid urine collection?

The main advantage of liquid urine collection is enhanced sensitivity, especially for low concentration metabolites. Dried urine must be reconstituted from the filter paper once the sample arrives. This reconstitution can lead to loss of polar steroid metabolites or creatinine for some patient samples. With liquid urine, samples can be shipped after being frozen for 4-6 hours, can be processed faster, and concentrated further to enhance the detection of low-level analytes. Steroids are also quite stable in liquid urine if the correct preservative is used.

Can this test be done for pregnant women?

While this test may be ordered during pregnancy, clinicians should understand urinary reference ranges during pregnancy have not been established.

Can I add neurotransmitter testing to the same urine sample?

Yes. Both the NeuroBasic and the Comprehensive Neurotransmitters profiles can be added to HuMap™ testing.

Many of the symptoms that would drive one to test urinary metabolite imbalance in a patient (fatigue, sleep difficulties, stress, mood concerns, cognitive concerns, vasomotor symptoms) can also be influenced by neurotransmitter imbalance. Additionally, the COMT (Catechol-O-Methyltransferase) enzyme plays an essential role in estrogen metabolism as well as catecholamine metabolism. Issues with COMT activity can result in both estrogen metabolite and neurotransmitter imbalance. Adding neurotransmitter testing to HuMap™ provides a deeper dive into the biochemistry that may be contributing to a patient's symptom picture.

What are unconjugated or free hormones?

Unconjugated/ free hormones are the main hormones found within the human body and are the hormones commonly measured in saliva or serum. Examples of unconjugated hormones are estradiol, testosterone, progesterone, and DHEA.

Are unconjugated hormones tested in urine?

Yes. Unconjugated hormones can be tested in urine, but it is more difficult to measure unconjugated/free sex hormone levels because of the biochemistry of these molecules. Progesterone, because of its proximity to cholesterol in the steroid cascade, is highly hydrophobic. The other sex hormones (estradiol, testosterone, etc.) are slightly more polar than progesterone enabling detection in urine, but only in very small amounts. When bioavailable hormone molecules travel to the liver, they are conjugated to a glucoronidate or a sulphate which makes them polar, allowing them to float freely in the urine. Thus, conjugated forms of hormones are readily found in urine, but it is not an ideal medium for measuring unconjugated/free hormones.

What are hormone metabolites?

Hormone metabolites are the breakdown products of unconjugated / free hormones. When bioavailable hormone molecules travel to the liver, they are conjugated to a glucoronidate or a sulphate which makes them polar, allowing them to float freely in the urine.

Do hormone metabolites have action within the body?

Hormone metabolites have physiologic action on their own; measuring them can also provide information on the activity of the enzymes that conjugate and prepare them for excretion. Measuring various hormone metabolites allows the practitioner to monitor phase 1 and phase 2 detoxification and assess the quantities of metabolites and how they may affect physiology.

How will a genetic COMT variant affect these results?

Genetic variations in COMT can affect the efficiency of the enzyme. This information can be seen in the estrogens section of the report or on the summary page in the key enzyme section. When COMT is slow, this can cause hydroxylated estrogens (ie 4-OH E1/E2) to back up, resulting in elevations. When this occurs, the patient can be at an increased risk for oxidative damage as hydroxylated metabolites are reactive and can form quinones or semi quinones if they are not properly methylated. Quinones cause DNA damage.

Are urinary hormones and their metabolites useful for monitoring hormone therapy?

Urinary hormone and metabolite testing is uniquely suited to provide insight into how hormones and their metabolites are moving through the body as well as risk assessment from the generation of certain metabolites. When using urinary metabolite testing, the level of detectable unconjugated sex hormone levels is not representative of circulating or bioavailable tissue hormone levels, because urine is not reflective of tissue uptake. When the goal of testing is to understand bioavailable levels of hormones, salivary testing may be a better option, especially if utilizing topical hormones.

How does BHRT/HRT affect urinary hormone and metabolite results?

Hormone therapy (BHRT / HRT) can influence unconjugated /free hormones as well as metabolites, depending on the route of administration. For more information, see instructions that come with each kit or refer to the first page of the "Best Practices for Specimen Collection".

Urine testing can overestimate the level of oral supplementation due to conjugation. Conjugates that are created when oral steroids are absorbed in the gut do not seem to have systemic effects, whereas when they pass through the liver, they may have systemic activity. For this reason, patients are asked to leave a dosage interval of 72 hours between oral hormone usage and urinary testing.

Urinary testing typically underestimates topically applied hormones because the pharmacokinetics of the way they are moving through the body and the way that they are absorbed does not form significant metabolites. Transdermal hormones may avoid first pass until there has been a great deal of tissue uptake, and so these hormones are less likely to be reflected in urine. While there may be a small rise in supplemented urinary hormone levels, it is unclear to what extent urine results correlate with clinical improvement.

Urine can be used to monitor the hormone metabolites produced from the delivery methods of pellets and intramuscular injections, but again, monitoring in urine may prove less accurate in determining dosage.

Urine testing provides additional insight into a complex subject. Whether measuring baseline levels, or monitoring a patient's utilization of hormone therapy, in every situation there are downstream metabolites that expand one's view.

Why are these results different than serum/saliva testing?

Steroid hormones (i.e. estradiol, testosterone, progesterone) are fat soluble molecules made with a cholesterol backbone (hydrophobic/lipophilic) that renders them insoluble in water. Because steroid hormones are hydrophobic, they must either be bound to a carrier protein that allows them to travel in water (such as in serum) or conjugated into metabolites, which are water soluble (as in urine). It's important to understand that while urine is water based, saliva is more favorable for measuring fat soluble hormones. When blood is filtered through the salivary glands, only the unbound/free hormones pass through and into the saliva. This concept is foundational to understanding the appropriate medium to test certain hormones.

If I have questions about the results, is there someone I can talk to?

Yes! Doctor's Data has clinical and scientific support available. Please call 1-800-323-2784 to request a consult.

Overview of Hormone Metabolism

Hormone metabolism is the process of taking both endogenous and exogenous hydrophobic substances (hormones) and changing them into hydrophilic molecules to be processed and excreted from the body. While most of this process relies heavily on enzymes located within the hepatocytes of the liver, enzymes for phase I, II, and III metabolisms are also found in extrahepatic tissues such as the adipose, intestine, kidney, lung, and skin. These reactions take place within the cells' cytoplasm, endoplasmic reticulum, and mitochondria.

Phase I reactions are typically facilitated by the cytochrome P450 family with many CYP subtypes, such as CYP1A1, CYP1B1, and CYP3A4. These reactions take place to make a substance more polar by adding functional groups like –OH. These reactions often create reactive metabolites requiring additional reactions in phase II to decrease their reactivity.

Phase II reactions further increase the polarity by the addition of hydrophilic groups via conjugation, glucuronidation, acetylation, or sulfation resulting in water-soluble products that can be excreted by the body. This step requires certain nutrients as well as enzymes like COMT and MTHFR.

Phase III is the final step which requires bile acids for effective elimination via the stool. When this step is slowed or not functioning optimally, metabolism is slowed. In some cases, as seen in estrogen metabolism, phase III metabolites can be reabsorbed and are not eliminated right away.

Almazroo, O. A., Miah, M. K., & Venkataramanan, R. (2017). Drug metabolism in the liver. Clinics in Liver Disease, 21(1), 1–20. https://doi.org/10.1016/j.cld.2016.08.001

Progesterones

Progesterone is produced by the corpus luteum following ovulation and to a lesser extent by the adrenal glands in both sexes. While found in the urine in small amounts, progesterone can be seen as a clinical marker of luteul activity and theraputic oral progesterone administration. The most important progesterone metabolite, pregnanediol (PDL), can serve as a urinary marker for endogenous progesterone levels and as an indicator of ovulation. PDL exists as two isomers, 5α-pregnanediol and 5β-pregnanediol. 5β-pregnanediol represents the majority end point of endogenous progesterone metabolism and appears to have little activity within the body, while 5α-pregnanediol, the lesser metabolite of PDL, can cross the blood brain barrier and may partially agonize GABA-A receptors. This action is possibly due to its role as an immediate precursor to allopregnanolone. Allopregnanolone is a potent neuroactive steroid capable of binding the GABA-A receptor often leading to sedative and anxiolytic action. The calming action of allopregnanolone is often seen with orally supplemented progesterone, as the liver metabolizes a large portion of oral progesterone to the neuroactive steroid allopregnanolone.

Progesterone (P4):

Elevated (males): In males, progesterone is produced in both the testes and adrenal glands. Elevated progesterone may be due to high dose pregnenolone supplementation, progesterone supplementation, exogenous progesterone exposure, increased HSD3A activity, reduced activity of CYP21A or CYP17A, and/or prostate pathology. In addition, elevations of both progesterone and pregnanediol, progesterone's major metabolite, have been reported in 21-hydroxylase deficiency.

Elevated (females): In cycling females, progesterone is primarily produced in the corpus luteum of the ovaries, and to a lesser degree in the adrenal glands. Menopausal females continue to produce small amounts of progesterone in the adrenal glands. Elevated levels of progesterone may be due to high dose pregnenolone supplementation, progesterone supplementation, exogenous progesterone exposure, pregnancy, disorders of luteinization, increased HSD3A activity, reduced activity of CYP21A or CYP17A, and rarely thecal cell tumors. In addition, elevations of both progesterone and pregnanediol, progesterone's major metabolite, have been reported in 21-hydroxylase deficiency.

Low (males): In males, progesterone is produced in both the testes and adrenal gland. Low/low range levels of progesterone may contribute to decreased sperm maturation and motility and may play a role in adverse prostate outcomes.

Low (females): In cycling females, progesterone is produced in the corpus luteum of the ovaries, and to a lesser extent the adrenal glands. Menopausal females continue to produce small amounts of progesterone in the adrenal glands. Low/low range levels of progesterone may be due to anovulation, amenorrhea, perimenopause and menopause.

5α-Pregnanediol (5A-PD):

Elevated: 5A-PD is a minor urinary metabolite of progesterone. Increased levels may be due to high levels of progesterone or pregnenolone, progesterone supplementation, or adrenocorticohyperplasia. 5A-PD may agonize GABA-A receptors.

Low (males): Lower levels of pregnanediol may be due to decreased progesterone or 5-alpha reductase activity.

Low (females): Lower levels of pregnanediol have been associated with amenorrhea, decreased ovarian function, PCOS, ovarian cancer, and certain complications of pregnancy.

5β-Pregnanediol (5B-PD)

Elevated (males): 5B-PD is the major progesterone metabolite. Increased levels may be due to high levels of progesterone and/or pregnenolone, progesterone supplementation, or adrenocorticohyperplasia. Elevations of both progesterone and pregnanediol have been reported in 21-hydroxylase deficiency.

Elevated (females): 5B-PD is the major progesterone metabolite. Increased levels may be due to high levels of progesterone and/or pregnenolone, pregnancy, ovarian cyst, pregnenolone and/or progesterone supplementation, or adrenocorticohyperplasia. In addition, elevations of both progesterone and pregnanediol have been reported in 21-hydroxylase deficiency.

Low (males): Lower levels of pregnanediol may be due to decreased progesterone or 5-beta reductase activity.

Low (females): Lower levels of pregnanediol are seen in cases of amenorrhea, decreased ovarian function, PCOS, ovarian cancer, and certain complications of pregnancy.

Allopregnanolone (ALLOP):

Elevated: Allopregnanolone is a downstream metabolite of progesterone considered a neurosteroid due to its ability to influence the GABA-A receptor, creating anxiolytic effects. Elevated levels can be seen with high endogenous progesterone as well as exogenous oral supplementation of progesterone.

Low (males): Low levels of allopregnanolone can be seen with low progesterone and decreased 5-alpha reductase or HSD3A activity.

Low (females): Low levels of allopregnanolone can be seen with low progesterone, anovulatory cycles, the use of oral contraceptives containing ethinyl estradiol and levonorgestrel, and decreased 5-alpha reductase HSD3A activity.

17-OH Progesterone (17-OHP):

Elevated (males): 17-OH Progesterone is the product of progesterone hydroxylation. Elevations are associated with idiopathic hirsutism, congenital adrenal hyperplasia, 11-beta-hydroxylase deficiency, adult onset viralizing adrenal hyperplasia, and in men with cytochrome P450c17 deficiency. Additionally, hyperinsulinemia and hyperglycemia (metabolic syndrome) push 17-hydroxylation of progesterone.

Elevated (females): 17-OH Progesterone is the product of progesterone hydroxylation. Elevations are associated with PCOS, idiopathic hirsutism, congenital adrenal hyperplasia, 11-beta-hydroxylase deficiency, and adult onset viralizing adrenal hyperplasia. Additionally, hyperinsulinemia and hyperglycemia (metabolic syndrome) push 17-hydroxylation of progesterone.

Low: There is very little research pertaining to low levels of this metabolite, making its clinical significance unknown. Low levels of the precursor hormones pregnenolone, 17-hydroxypregnenolone, and progesterone can contribute to decreased levels.

21-Hydroxyprogesterone (21-OHP):

Elevated: 21-Hydroxyprogesterone is a steroid hormone with mineralocorticoid properties produced in the adrenal gland which serves as a precursor hormone to aldosterone. Elevated levels may cause mineralocorticoid hypertension. Elevations have been associated with chronic exposure to ACTH, Cushing's disease, type 2 diabetes, congenital adrenal hyperplasia and adrenocortical carcinoma.

Low: 21-Hydroxyprogesterone is a steroid hormone with mineralocorticoid properties produced in the adrenal gland which serves as a precursor hormone to aldosterone. Low levels maybe the result of low progesterone and/or primary or secondary adrenal insufficiency.

Pregnenetriol (5-PT):

Elevated: Pregnenetriol is a metabolite of 17α-pregnenolone, an intermediary resulting from the hydroxylation of pregnenolone by CYP17A1 enzyme. Elevations in urine may be seen in cases of Cushing's Syndrome, congenital adrenal hyperplasia, and adrenocortical carcinoma.

Low: Pregnenetriol is a metabolite of 17α-pregnenolone, an intermediary resulting from the hydroxylation of pregnenolone by CYP17A1 enzyme. Lower levels may be a result of deficiency of CYP17A1 activity.

5A-PD : 5B-PD

The metabolic prioritization for alpha or beta reductase activity within the progesterone pathway may be confirmatory of a general preference of metabolism. Comparing these results with the metabolic preference of androgens and corticoids may provide additional insight.

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Corticoids

The corticoid pathway has two main branches: glucocorticoids and mineralocorticoids. While the roles of these pathways vary, they share a common enzyme, CYP21A2, also known as 21- hydroxylase. 21-hydroxylase is part of the cytochrome P450 system and is responsible for the conversion of progesterone to 21-hydroxyprogesterone in the mineralocorticoid pathway. In the glucocorticoid pathway it converts 17-OH progesterone to pregnanetriol, 11-deoxycortisol, and 21 deoxycortisol. 11-deoxycortisol and 21-hydroxyprogesterone are metabolized by CYP11B (11-beta hydroxylase) enzyme to produce cortisol and corticosterone, respectively. The major site of cortisol metabolism is the liver. There it is reduced, oxidized or hydroxylated. The enzymes that directly metabolize cortisol are 11 beta hydroxyosteroid dehydrogenase 1 and 2 (11BHSD1 and 11BHSD2), the A-ring reductases (5 alpha and 5 beta reductases), 3 alpha hydroxysteroid dehydrogenase and 20 alpha and 20 beta hydroxysteroid dehydrogenases.

The clearance of active cortisol from circulation is largely affected by 11BHSD1 and 2 activity. Cortisone protects tissues from the effects of cortisol, therefore if 11BHSD activity is functioning properly, there should be twice as much cortisone as cortisol, measured in the cortisone (THE)/cortisol (THF) ratio. This ratio indicates 11BHSD 2 activity and infers tissue-specific concentrations of cortisol (which normally cannot be measured without a biopsy). A lower ratio suggests decreased cortisol metabolism/clearance by 11BHSD2, indicating an increased cortisol burden on tissues; whereas a higher ratio reflects optimal 11BHSD2 activity.

HSD 11B (1 and 2)

The primary function of 11BHSD2 is to protect cells with mineralocorticoid receptors (MR) from excessive cortisol by converting it to cortisone. Since cortisol has the same affinity for MR as aldosterone and is present in much higher concentrations, the conversion of cortisol to cortisone protects cells from glucocorticoid intrusion on the mineralocorticoid system. It has been shown that the cortisol pool results not only from the production of cortisol through classic HPA Axis processes, but also from the actions of 11BHSD1 due to the re-conversion of cortisone back to cortisol. The human liver/splanchnic bed is responsible for only 20-40% of daily cortisol production rendering the inactive cortisone pool a major reservoir for systemic cortisol availability. For this reason, in cells expressing 11BHSD1, cortisone is considered just as potent as cortisol (highest in liver, adipose, CNS, skeletal muscle and the immune system).

Those with metabolic disorders (such as metabolic syndrome, obesity, diabetes, CVD, cognitive disorders, bone disorders and inflammation) may not present with elevations of serum or salivary free cortisol, yet adipose tissue has been shown to have higher levels of 11BHSD1 than controls, suggesting elevated glucocorticoid activity may be taking place despite free cortisol labs suggesting otherwise. Pro-inflammatory cytokines have been shown to upregulate 11BHSD1 enabling a tissue-specific cortisol induced anti-inflammatory response. This effect has also been found in chronic disease such as obesity, cardiovascular and neurodegenerative diseases, and bone and joint disorders.

Thyroid hormone plays a role in this conversion process. Increased cortisol metabolism resulting in increased cortisone is associated with hyperthyroidism. Hypothyroidism has been shown to slow cortisol metabolism, resulting in lower levels of metabolized cortisol.

When evaluating the corticoids it is important to pay attention to three areas on the HuMap™.

1. Graphical pattern of daily cortisol and cortisone
2. Metabolized Cortisol
3. Metabolic preference for cortisol and cortisone

Corticosterone (B):

Elevated/Low: Corticosterone is a precursor hormone to aldosterone. Research is limited in the clinical significance of both elevated or low corticosterone and may be due to levels of precursor hormones.

Tetrahydrodehydrocorticosterone (THA):

Elevated/Low: 5B-THA is a terminal metabolite of corticosterone. This metabolite in combination with other terminal metabolites can be used to estimate metabolism of corticosterone. While research in elevations or low levels of single terminal metabolites is limited, assessing metabolism may provide valuable information about enzyme activity.

5β-Tetrahydrocorticosterone (5B-THB):

Elevated/Low: 5B-THB is a terminal metabolite of corticosterone. This metabolite in combination with other terminal metabolites can be used to estimate metabolism of corticosterone. While research in elevations or low levels of single terminal metabolites is limited, assessing metabolism may provide valuable information about enzyme activity.

5α-Tetrahydrocorticosterone (5A- THB):

Elevated/Low: 5A-THB is a terminal metabolite of corticosterone. This metabolite in combination with other terminal metabolites can be used to estimate metabolism of corticosterone. While research in elevations or low levels of single terminal metabolites is limited, assessing metabolism may provide valuable information about enzyme activity.

11-Deoxycortisol (11-DOC)

Elevated: 11-Deoxycortisol has very little glucocorticoid activity yet it's helpful to understand its role as an intermediate in cortisol creation and how it can contribute to impairment of the pathway. 11-Deoxycortisol is metabolized via CYP11B (11-beta hydroxylase) to cortisol. Elevations of 11-deoxycortisol maybe due to impairment of CYP11B, congenital adrenal hyperplasia, or andrenocortico tumors. Elevations of blood pressure due to a buildup of 11-deoxycortisol have been reported.

Low: 11-Deoxycortisol has very little glucocorticoid activity yet its role as an intermediate in cortisol creation may assist in understanding of impairment along this pathway. CYP21A (21-hydroxylase) is responsible for the conversion of 17-hydroxyprogesterone to 11-deoxycortisol. 21-hydroxylase deficiency can lead to a decrease in the production of 11-deoxycortisol. A complete understanding of the corticoids may provide more clinical information.

Cortisol (F):

Elevated (males): Cortisol is the main glucocorticoid released from the adrenal gland in response to stress. High levels of cortisol have been reported in cases of Cushing's disease, malnutrition, early life stress, hypothyroidism, depression, alcoholism, obesity, and critical illness. Additionally, exogenous exposure to glucocorticoids prior to testing may be a source of cortisol elevations.

Elevated (females): Cortisol is the main glucocorticoid released from the adrenal gland in response to stress. High levels of cortisol have been reported in cases of Cushing's disease, malnutrition, early life stress, hypothyroidism, depression, alcoholism, PCOS, obesity, and critical illness. Additionally, exogenous exposure to glucocorticoids prior to testing may be a source of cortisol elevations.

Low: Low cortisol levels may be due to low production or excessive metabolism by 11BHSD as seen in obesity. Very low levels of cortisol have also been reported in Addison's disease.

Cortisone (E):

Elevated: Cortisone is the inactive form of cortisol. Elevations of cortisone may reflect high cortisol production, excessive 11BHSD2 activity, or insufficient conversion by 11BHSD1.

Low: Cortisone is the inactive form of cortisol. Low levels may reflect low cortisol production, excess conversion by 11BHSD1, or insufficient 11BHSD2 activity.

5α-Tetrahydrocortisol (5A-THF)

Elevated: 5A-THF is a terminal metabolite of cortisol metabolized via 5-alpha reductase. Combining all the terminal metabolites can be used to estimate metabolized cortisol. While research into single terminal metabolite elevations is limited, it may have more clinical relevance when assessed in combination with the daily output of free cortisol.

Low: 5A-THF is a terminal metabolite of cortisol metabolized via 5-alpha reductase. This metabolite along with the other terminal metabolites can be used to determine metabolized cortisol. Research in low levels of a single terminal metabolites is limited, assessing metabolized cortisol and daily output may provide more clinically relevant information.

5β-Tetrahydrocortisol (5B-THF)

Elevated: 5B-THF is a terminal metabolite of cortisol metabolized via 5-beta reductase. This metabolite along with the other terminal metabolites can be used to determine metabolized cortisol. While research in elevations of single terminal metabolites is limited, assessing metabolized cortisol and daily output may provide more clinically relevant information.

Low: 5B-THF is a terminal metabolite of cortisol metabolized via 5-beta reductase. This metabolite along with the other terminal metabolites can be used to determine metabolized cortisol. Research in low levels of single terminal metabolites is limited, assessing metabolized cortisol and daily output may provide more clinically relevant information.

Tetrahydrocortisone (THE)

Elevated/Low: THE is a terminal metabolite of cortisone. This metabolite along with the other terminal metabolites can be used to determine metabolized cortisol. Research in elevations or low levels of single terminal metabolites is limited, assessing metabolized cortisol and daily output may provide more clinically relevant information.

Ratios and Calculations

DHEA + DHEAS

DHEA and DHEAs are produced in the adrenal gland and serve as precursors to androgens and estrogens. Due to the interconversion between DHEA and DHEAS via SULT2A1 and/or STS, the sum of these may be a better representation of total DHEA synthesis.

THE+5A-THF+5B-THF (Total Cortisol Metabolites)

Elevated: This calculation includes the daily metabolites of cortisol (5-alpha THF, THF) and cortisone (THE) which maybe a better representation of daily cortisol output than measuring cortisol and cortisone alone due to metabolism differences in the liver (with thyroid hormone) and fatty tissues. High levels can indicate increased cortisol secretion or hyperthyroidism.

Low: This calculation includes the daily metabolites of cortisol (5-alpha THF, THF) and cortisone (THE) which maybe a better representation of daily cortisol output than measuring cortisol and cortisone alone due to metabolism differences in the liver (with thyroid hormone) and fatty tissues. Low levels may indicate decreased cortisol secretion or hypothyroidism.

5A-THF+5B-THF/THE (Cortisol/Cortisone Metabolites)

Elevated (males): The relationship of the cortisol metabolites (5-alpha THF + THF) to cortisone metabolite (THE) is another tool in the assessment of the dominance of active cortisol or inactive cortisone. An elevated ratio means suppressed enzyme activity or low conversion rate of cortisol to cortisone. This can be seen in stress, hypertension, metabolic syndrome, insulin resistance, depression, with cortisol supplementation, or high licorice dosages.

Elevated (females): The relationship of the cortisol metabolites (5-alpha THF + THF) to cortisone metabolite (THE) is another tool in the assessment of the dominance of active cortisol or inactive cortisone. An elevated ratio means suppressed enzyme activity or low conversion rate of cortisol to cortisone. This can be seen in stress, hypertension, metabolic syndrome, insulin resistance, PCOS, depression, with cortisol supplementation or high licorice dosages.

Low: The relationship of the cortisol metabolites (5-alpha THF + THF) to cortisone metabolite (THE) is another tool in the assessment of the dominance of active cortisol or inactive cortisone. A low ratio reflects a higher conversion rate of cortisol to cortisone which can be normal in some cases, or may be due to overt or subclinical thyroid pathology.

Cortisol/Cortisone (11B HSD activity)

Elevated (males): Cortisol / cortisone ratio reflects HSD11B2 activity and assessment of tissue specific concentrations of cortisol, which normally cannot be measured without a biopsy. An elevated ratio indicates suppressed enzyme activity or a low conversion rate of cortisol to cortisone. This can be seen in stress, hypertension, metabolic syndrome, insulin resistance, depression, with cortisol supplementation, or high dose licorice supplementation.

Elevated (females): Cortisol / cortisone ratio reflects HSD11B2 activity and assessment of tissue specific concentration of cortisol, which normally cannot be measured without a biopsy. An elevated ratio indicates suppressed enzyme activity or a low conversion rate of cortisol to cortisone. This can be seen in stress, hypertension, metabolic syndrome, insulin resistance, PCOS, depression, with cortisol supplementation, or high licorice doses.

Low: Cortisol / cortisone ratio indicates activity of HSD11B2 activity and assessment of tissue specific concentrations of cortisol, which normally cannot be measured without a biopsy. A low ratio reflects a higher conversion rate of cortisol to cortisone, which can be normal in some cases. Hyperthyroidism can also be a cause of a lowered cortisol/cortisone ratio.

5A-THF/5B-THF ratio (alpha vs beta metabolism)

Elevated/Low: The 5A-THF/5B-THF ratio is a calculation used to show the preference of 5-alpha reductase activity to 5-beta reductase activity. While research is limited in the significance of 5-alpha or 5-beta reductase activity in the glucocorticoids, it can serve as an additional screening tool for overall preference for 5-alpha or 5-beta reductase activity within the androgen and progesterone pathways.

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Androgens

Androgens play a significant role in structure and function of muscle, bone, and connective tissue, metabolic homeostasis and reproduction in both men and women. When evaluating the androgens, it is important to look at parent hormones, enzymes, metabolites, and clinical symptoms to gain an understanding of the complete clinical picture.

The key areas of focus within the androgen pathway are androstenedione, DHEA, testosterone, 5-alpha and 5-beta reductase, and aromatase (CYP19). Testosterone is derived from androstenedione and DHEA. 5-alpha reductase converts testosterone into the metabolite 5α-DHT which is three times more potent than testosterone. Symptoms associated with higher androgen levels (thinning hair, acne, etc) are often seen when levels of 5-alpha reductase and its corresponding metabolites are elevated. 5-beta reductase and its corresponding metabolites are much less androgenic. The assessment of 5-alpha and 5-beta metabolisms can be understood by following the metabolism of androstenedione to etiocholanolone (5β) and androstenedione to androsterone (5α). The relative ratio between these two pathways and the amounts of 5α-androstanediol and 5β-androstanediol may give insight into the full androgenic picture. Androgen deficiency symptoms can be caused by lower levels of DHEA, testosterone, or 5α metabolites.

Androgens are also precursors to estrogens. The enzyme aromatase (CYP19) helps to convert androstenedione to estrone and testosterone to estradiol. This enzyme is the most active within peripheral fat tissues of both males and females. Understanding the connection between aromatase and the estrogen pathway may give additional clinical insight into patients' symptoms.

Androstenedione (A4)

Elevated (males): Androstenedione is a weak androgen secreted by adrenal glands, testes, and ovaries that is converted to testosterone and/or estrone in the periphery. Research suggests elevations of androstenedione can aromatize to estrogens and may be associated with gynecomastia and testicular atrophy. The production of adrenal androstenedione is governed by ACTH, while gonadal androstenedione is influenced by gonadotropins.

Elevated (females): Androstenedione is a weak androgen secreted by adrenal glands, testes, and ovaries that is converted to testosterone or estrone in the periphery. Elevated levels are correlated with PCOS, insulin resistance, hirsutism, amenorrhea, and acne. The production of adrenal androstenedione is governed by ACTH, while gonadal androstenedione is influenced by gonadotropins.

Low: Androstenedione is a weak androgen secreted by adrenal glands, testes, and ovaries that is converted to testosterone or estrone in the periphery. Low levels of this androgen precursor may correlate with symptoms of androgen deficiency. The production of adrenal androstenedione is governed by ACTH, while production of gonadal androstenedione is influenced by gonadotropins.

EPI-Testosterone (EPI-T)

Elevated: This epimer of testosterone is produced in equal amounts with testosterone. It is also a weak competitive antagonist of the androgen receptor making it a weak antiandrogen. Epi-T can inhibit 5-alpha reductase. Measurement of epi-T in urine is used to detect athletic doping since epi-T is unaffected by exogenous testosterone supplementation. Ingestion of alcohol has been shown to increase epi-T levels.

Low (males): Low levels of epitestosterone may be the result of low precursor levels of testosterone. Research is limited in the significance of this finding.

Low (females): Low levels of epitestosterone may be the result of low precursor levels and/or hormonal contraceptives. Research is limited in the significance of this finding.

Testosterone (T)

Elevated (males): Testosterone in supplementing males can be associated with acne, hair loss, anger, anxiety, fatigue, etc. Testosterone products should not be used in men contemplating or attempting to initiate pregnancy. (It is important to monitor hematocrit while supplementing with testosterone and consider a PSA and rectal exam before initiating therapy.)

Elevated (females): Testosterone is the major anabolic androgen found in females. Because urinary testosterone levels are independent of circadian rhythm fluctuation, urine maybe a better medium than serum to indicate androgen production. Elevated urinary testosterone levels have been associated with insulin resistance, metabolic syndrome, increased visceral fat, congenital adrenal hyperplasia, PCOS with hirsutism, idiopathic hirsutism, Cushing Syndrome, and masculinizing adrenal adenoma. It's also associated with insulin resistance, metabolic syndrome, and increased visceral fat. It's important to rule out exogenous exposure, especially in a household where another member is using a topical testosterone supplement.

Low (males): Testosterone is the major anabolic androgen found in males. Because urinary testosterone levels are independent of circadian rhythm fluctuation, urine maybe a better medium than serum to indicate androgen production. Low levels of testosterone can be seen in hypogonadism along with associated symptoms of erectile dysfunction, low libido, depression, infertility, hot flashes and osteoporosis. Hypogonadism can also be associated with anemia, gynecomastia, depressed mood, diminished bone density, low energy, decreased muscle mass and performance, hot flashes/sweats, impaired cognition, increased BMI, infertility, hair loss, decreased libido and sexual function, and hypospadias. Note: Testosterone products should not be used in men contemplating or attempting to initiate pregnancy.

Low (females): Testosterone is the major anabolic androgen found in females. Because urinary testosterone levels are independent of circadian rhythm fluctuation, urine may be a better medium than serum to indicate androgen production. Low levels of testosterone as well as other androgens can lead to symptoms of low libido, depression, decreased muscle size, and strength.

Androsterone (AN)

Elevated (males): Androsterone is the product of androgens metabolized by 5-alpha reductase. It acts as a neurosteroid and a weak potentiator of GABA-A receptor activity. Androsterone may also be converted to DHT via backdoor pathway using HSD3B and HSD17B making it a metabolic intermediate. Potential causes of elevation may include over supplementation of DHEA or pregnenolone, androgen producing gonadal tumors, congenital adrenal hyperplasia, adult-onset adrenal hyperplasia, serious illness, shock, and burns.

Elevated (females): Androsterone is the product of androgens metabolized by 5-alpha reductase. It acts as a neurosteroid and a weak potentiator of GABA-A receptor activity. Androsterone may also be converted to DHT via backdoor pathway using HSD3B and HSD17B making it a metabolic intermediate. Potential causes of AN elevation may include PCOS, over supplementation of DHEA or pregnenolone, androgen producing gonadal tumors, congenital adrenal hyperplasia, adult-onset adrenal hyperplasia, serious illness, shock, and burns.

Low: Androsterone is the product of androgens metabolized by 5-alpha reductase. It acts as a neurosteroid and a weak potentiator of GABA-A receptor activity. Inhibiting 5-alpha reductase lowers AN. 24-hour urine testing has shown that AN declines along with DHEA. Low levels may also be associated with adrenal insufficiency, anorexia nervosa and panhypopituitarism.

11-Hydroxy-Androsterone (OHAN)

Elevated (males): OHAN is a urinary metabolite of cortisol metabolism as well as 11-oxygenated androgens production from the adrenal glands. While research is limited in the significance of elevations of this metabolite, it may be associated with certain conditions like 21-hydroxylase deficiency and castration-resistant prostate cancer.

Elevated (females): OHAN is a urinary metabolite of cortisol metabolism as well as 11-oxygenated androgens production from the adrenal glands. While research is limited in the significance of elevations of this metabolite, it may be associated with certain conditions like PCOS and 21-hydroxylase deficiency.

Low: OHAN is a urinary metabolite of cortisol metabolism as well as 11-oxygenated androgens production from the adrenal glands. Low levels maybe reflective of low adrenal androgen production.

5α-Androstanediol (5A-AD)

Elevated (males): 5A-AD is a metabolite of 5αDHT. Research in elevations of this metabolite is limited in males.

Elevated (females): 5A-AD is a metabolite of 5αDHT. Research suggests elevations of this pathway in females maybe due to PCOS and hirsutism.

Low (males): 5A-AD is a metabolite of 5αDHT. It has been shown to be significantly reduced with finasteride or medications decreasing 5-alpha reductase.

Low (females): 5A-AD is a metabolite of 5αDHT. Research suggests that postmenopausal women may experience low levels of this metabolite.

5α-Dihydrotestosterone (5A-DHT)

High (males): 5A-DHT is converted from testosterone by 5-α reductase in the testes and prostate. Higher DHT levels may be associated with truncal obesity in males, androgenic alopecia, sexual dysfunction, alterations in mood and body composition, and testosterone supplementation. DHT promotes cell growth and may play a role in prostate issues.

High (females): 5A-DHT is converted from testosterone by 5-α reductase in the ovaries and peripherally in fat tissue. Higher levels may be associated with acne, scalp hair loss, and hirsutism.

Low (males): 5A-DHT is converted from testosterone by 5-α reductase in the testes and prostate. Low levels may result from low testosterone or prostatectomy.

Low (females): 5A-DHT is converted from testosterone by 5-α reductase in the ovaries and peripherally in fat tissue. Low levels may cause symptoms of low androgens: thinning skin, low libido, vaginal dryness, depression, and fatigue.

Etiocholanolone (ET)

Elevated (males): Etiocholanolone is a 5-beta reduced isomer of androsterone, and a major metabolite of testosterone and androstenedione, however it is not active as an androgen. Excessive levels maybe the result of DHEA supplementation and is associated with androgenic alopecia.

Elevated (females): Etiocholanolone is a 5-beta reduced isomer of androsterone, and a major metabolite of testosterone and androstenedione, however it is not active as an androgen.

Low: Etiocholanolone is a 5-beta reduced isomer of androsterone, and a major metabolite of testosterone and androstenedione, however it is not active as an androgen. Low levels suggests that the 5-alpha pathway may be outcompeting the 5-beta pathway in androgen metabolism.

11-Hydroxy-Etiocholanolone (OHET)

Elevated: OHET is the product of cortisol metabolism as well as 11-oxygenated androgens produced from the adrenal gland. Levels tend to reflect levels of etiocholanolone.

Low: OHET is the product of cortisol metabolism as well as 11-oxygenated androgens produced from the adrenal gland. Levels tend to reflect levels of etiocholanolone.

5β-Androstanediol (5B-AD)

Elevated: 5B-AD is the result of the 5-beta reduction of DHT and is a metabolite of etiocholanolone. High levels may be due to an increased conversion via 5-beta reductase, or from DHEA or testosterone supplementation.

Low: 5B-AD is the result of 5-beta reduction of DHT as well as a metabolite of etiocholanolone. May result from low levels of DHEA or testosterone or lower activity 5-beta reductase.

Dehydroepiandrosterone (DHEA)

Elevated (males): Dehydroepiandrosterone (DHEA) is predominantly produced in the adrenal glands and serves as a precursor hormone for androstenedione and eventually estrone and testosterone. High levels of DHEA may be due to DHEA or pregnenolone supplementation. Additional research suggests DHEA elevations may also be due to such conditions as adult-onset adrenal hyperplasia, congenital adrenal hyperplasia, and very rarely, adrenal carcinoma. SULT2A1 catalyzes the sulfate conjugation of DHEA, and research suggests dopamine can induce this enzyme.

Elevated (females): Dehydroepiandrosterone (DHEA) is a hormone predominantly produced in the adrenal glands which serves as precursor hormone for androstenedione and eventually estrone and testosterone. High levels of DHEA may be a result of the use of pregnenolone or DHEA supplementation. Additional research suggests DHEA elevations may also be due to PCOS, adult-onset adrenal hyperplasia, congenital adrenal hyperplasia, and very rarely adrenal carcinoma.

Low: Dehydroepiandrosterone (DHEA) is a hormone predominantly produced in the adrenal glands which serves as precursor hormone for androstenedione and eventually estrone and testosterone. DHEA naturally declines with age and under the influence of chronic and sub-chronic stress. Research suggests that low DHEA can manifest in cognitive decline; changes in libido, mood, and flexibility; and cardiovascular health.

Dehydroepiandrosterone Sulfate (DHEAS)

Elevated (males): Dehydroepiandrosterone sulfate or DHEA-S is the sulfated form of dehydroepiandrosterone (DHEA) and the major steroid precursor in humans. This sulfation is reversibly catalyzed by sulfotransferase 2A1 (SULT2A1) primarily in the adrenals, the liver, and the small intestine. Like DHEA, research suggests DHEA-S elevations could be due to adult-onset adrenal hyperplasia, congenital adrenal hyperplasia, and very rarely adrenal carcinoma. Increased levels of DHEA, as well as pregnenolone, through either supplementation or endogenous excretion, may also contribute to elevated levels of DHEAS.

Elevated (females): Dehydroepiandrosterone sulfate or DHEA-S is the sulfated form of dehydroepiandrosterone (DHEA) and the major steroid precursor in humans. This sulfation is reversibly catalyzed by sulfotransferase 2A1 (SULT2A1) primarily in the adrenals, the liver, and the small intestine. Like DHEA, research suggests DHEA-S elevations could be due PCOS, adult-onset adrenal hyperplasia, congenital adrenal hyperplasia, and very rarely adrenal carcinoma. Increased levels of DHEA, as well as pregnenolone, through either supplementation or endogenous excretion, may also contribute to elevated levels of DHEAS.

Low: Dehydroepiandrosterone sulfate (DHEA-S), the sulfated form of dehydroepiandrosterone (DHEA), is primarily produced by the zona reticularis of the adrenal glands and serves as a reservoir for DHEA. Like DHEA, DHEA-S naturally declines with age. Research suggests symptoms of declining DHEA-S can manifest as declining cognition, libido, mood, flexibility, and cardiovascular health.

Ratios and Calculations

DHEA + DHEAS

DHEA and DHEAs are produced in the adrenal gland and serve as precursors to androgens and estrogens. Due to the interconversion between DHEA and DHEAS via SULT2A1 and/or STS, the sum of these maybe a better representation of total DHEA synthesis.

AN:ET (alpha vs beta metabolism)

Elevated (males): This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Elevated levels may indicate higher 5-alpha reductase activity which may lead to an increase in androgenic symptoms.

Elevated (females): This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Elevated levels may indicate higher 5-alpha reductase activity which may lead to an increase in androgenic symptoms like hirsutism and scalp hair loss.

Low: This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Decreased levels may be due to the use of 5-alpha reductase inhibition medication or inherent preference for 5-beta metabolism.

T:EPI-T

Elevated (males): The T/Epi-T ratio should be around 1. Elevations in the T/Epi-T ratio in males can be caused by exogenous testosterone supplementation.

Elevated (females): The T/Epi-T ratio should be around 1. Elevations in the T/Epi-T ratio in females can be caused by exogenous testosterone supplementation and in some cases hormonal contraceptives which lower epi-T increasing T/Epi-T ratio.

Low: The T/Epi-T ratio should be around 1. A low T/Epi-T ratio may be decreased due to low levels of testosterone. Research is limited in the significance of low T/Epi-T ratio in males and females.

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Estrogens

Evaluation of the estrogen metabolism pathway relies on understanding several key steps of metabolism: the amount of unconjugated estrogens, hydroxylation of E1 and E2, methylation of hydroxy estrogens, and the function of key enzymes.

Unconjugated Estrogens:

The unconjugated estrogens are the main steroid hormones estrone (E1), estradiol (E2), and estriol (E3). These hormones are primarily produced in and excreted from the gonads (ovaries and testes), with a smaller percentage coming from the adrenal glands and conversion in peripheral tissues. The amount of unconjugated estrogens is important as this will determine the starting pool for further metabolism.

Hydroxylation of Estrogens:

Phase I metabolism is essentially the addition of a reactive hydroxyl group to the 2 and 4 positions of estrone and estradiol and the 16 position of estrone. These estrogens make up what is known as the catechol estrogens. 2-OH E1 and 2-OH E2 are the primary metabolites of the estrogens and are thought to be "safe" due to their low potencies, association with cell differentiation, high clearance rate, and anti-cancer properties compared to the 4-OH pathway metabolites. The 4-OH pathway tends toward "riskier" behavior as these metabolites can generate a large amount of free radical and DNA damage compared to the 2-OH pathway. 16-OH E1 is considered the most "estrogenic" of the metabolites as it can be hydroxylated to estriol, a non-proliferative / protective estrogen, and has also been shown to play a role in genotoxic reactions. The enzyme HSD17B is important to estradiol metabolism as it converts 16α-OHE1 to E3, E1 to E2, and E2 to E1. The ratio of 2OHE1 to 16a-OHE1 can provide a marker for breast health and cancer risk with a lower 2OHE1 to 16a-OHE1 ratio shown to correlate with higher cancer risk.

When evaluating phase 1 metabolism, comparison of 2, 4, and 16 hydroxy metabolites may elucidate which pathways are preferred. Understanding this can be essential in choosing the appropriate treatments.

Methylation of Hydroxy Estrogens:

Phase II detoxification of 2-OH and 4-OH metabolites via Catechol-o-Methyl Transferase (COMT) creates 2-M E1/E2 and 4-M E1/E2. Methyl metabolites are harmless and, in this form, can be rapidly excreted in the urine. If methylation pathways are inadequate due to low levels of COMT or cofactors necessary for methylation (Magnesium, SAMe), or deficiencies in folate, B6, B12, or MTHFR genetic defects, the 2-OH and 4-OH metabolites can travel down a more metabolically dangerous pathway leading to oxidation and the potential for the formation of highly reactive quinones. Estrogen quinones, especially the 4-quinone of E1 and E2, are highly reactive and can bind to DNA to form adducts that can lead to permanent mutations in DNA. Estrogen quinones are rendered inactive when bound to glutathione. Adequate glutathione levels depend upon sufficient levels of selenium, iodine, vitamin C and E. Without adequate levels of glutathione, 2- and 4 quinones may not be detoxified, carrying the potential for irreparable damage to cells and DNA.

The ratio of 4-M E1/E2 to 4-OH E1 / 2 and 2-M E1/E2 to 2-OH E1/E2 can help determine if adequate methylation of catechol estrogens is occurring. The higher the ratio, the higher the likelihood of metabolizing toward the less harmful pathway of methylation and therefore less reactive quinone formation. Even if 4-OH metabolites are elevated, adequate methylation means these metabolites are being detoxified rendering them less harmful.

Estrone (E1)

Elevated (males): A component of the estrone level may be due to aromatization of androstenedione and testosterone by CYP19 (aromatase) enzyme in adipose tissue, and/or conversion from estradiol due to HSD17B activity. Aromatase up-regulation and increased intracellular estrogens in men may contribute to increased adiposity, metabolic syndrome, and prostate pathology. CYP19 enzyme is induced during times of stress, exposure to xeno-estrogens, high glycemic diet, excessive adipose tissue, and alcohol consumption.

Elevated (females): A component of the estrone level may be due to aromatization of androstenedione and testosterone by CYP19 (aromatase) enzyme in adipose tissue and/or conversion from estradiol due to HSD17B activity. Elevated estrone has been associated with increased risk of breast cancer in postmenopausal women, particularly when accompanied by elevated testosterone. CYP19 enzyme is induced during times of stress, exposure to xeno-estrogens, high glycemic diet, excessive adipose tissue, and alcohol consumption.

Low: Has not been shown to be associated with negative health effects.

2-Hydroxyestrone (2-OH-E1)

Elevated (males): Adequate levels of 2-OH-E1 have been shown to be a favorable marker for breast health in women, and while research in men in this area is lacking, the health benefits may be the same for men. Elevated levels can be due to high endogenous estrone as well as exogenous exposure to or supplementation of estrone and/or estradiol. While 2-OH-E1 is considered the "safer" estrogen metabolite, optimizing methylation to support the COMT enzyme will ensure favorable excretion rates.

Elevated (females): Adequate levels of 2-OH-E1 have been shown to be a favorable marker for breast health. Elevated levels can be due to high endogenous estrone as well as exogenous exposure to or supplementation of estrone and/or estradiol. While 2-OH-E1 is considered the "safer" estrogen metabolite, optimizing methylation to support the COMT enzyme will ensure favorable excretion rates.

Low (males): Adequate levels of 2-OH-E1 have been shown to be a favorable marker for breast health in women, and while research in men in this area is lacking, the health benefits may remain the same for men. Low levels of 2-OH-E1 may be due to low levels of estrone, or more active CYP3A4 or CYP1B1 enzymes. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

Low (females): Adequate levels of 2-OH-E1 have been shown to be a favorable marker for breast health. Low levels of 2-OH E1 may be due to low levels of estrone, or more active CYP3A4 or CYP1B1 enzymes. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

4-Hydroxyestrone (4-OH-E1)

Elevated (males): Higher levels indicate slowed COMT activity (methylation) and are associated with a higher risk for breast cancer in females. Due to the lack of research in this area, it may be postulated that men carry a similar risk. Elevation may also be due to an overactive CYP1B1 enzyme or sluggish CYP1A1 or CYP34A. Additional support for the COMT enzyme can help with the conversion toward the inactive metabolite, 4-M-E1.

Elevated (females): Higher levels indicate slowed methylation and are associated with a higher risk for breast cancer in women. Elevation may also be due to an overactive CYP1B1 enzyme or sluggish CYP1A1 or CYP34A. Additional support for the COMT enzyme can help with the conversion toward the inactive metabolite, 4-M-E1.

Low (males): 4-OH E1 is associated with a higher risk of certain cancers and other markers for breast health in women. Due to the lack of research in men, it may be postulated that men carry similar risks. Low levels may be beneficial depending on the other metabolites and methylation further down the pathway.

Low (females): 4-OH E1 is associated with a higher risk of certain cancers and other markers for breast health in women. Low levels may be beneficial depending on the other metabolites and methylation further down the pathway.

16α-Hydroxyestrone (16-OH-E1)

Elevated (males): Higher levels of 16-OH-E1 are associated with a higher risk of certain cancers and other negative markers of breast health in females. Due to lack of research in this area for men, it can be postulated that males carry a similar risk. Elevations in 16-OH-E1 may be due to increased metabolism from estrone or a sluggish HSD17B enzyme, keeping 16-OH-E1 from converting into estriol.

Elevated (females): Higher levels of 16-OH-E1 are associated with a higher risk of certain cancers and other negative markers of breast health in females. Elevations in 16-OH-E1 may be due to increased metabolism from estrone or a sluggish HSD17B enzyme, keeping 16-OH-E1 from converting into estriol.

Low: Lower levels of 16-OH-E1 are associated with a lower risk of certain cancers. Low levels may be due to low levels of unconjugated estrogens. Evaluation of unconjugated estrogens for supplementation may be necessary to regain normal levels of 16-OH-E1.

2-Methoxyestrone (2-M-E1)

Elevated: 2-M-E1 is considered a non-reactive metabolite. Higher levels have been correlated with antiproliferative and antiangiogenic effects as well as cardioprotective properties. Depending on other metabolite values and optimal GI function and excretion, elevations in 2-M-E1 may be considered favorable.

Low (males): 2-M-E1 is considered a non-reactive metabolite. Lower levels have been associated with a higher risk of certain cancers and other markers for breast health in women. Due to the lack of research in this area, it may be postulated that men carry similar risks. A genetic variant of the MTHFR enzyme may contribute to decreased methylation. If a variant is suspected, further evaluation may be warranted.

Low (females): 2-M-E1 is considered a non-reactive metabolite. Lower levels have been associated with a higher risk of certain cancers and other markers for breast health. A genetic variant of the MTHFR enzyme may contribute to decreased methylation. If a variant is suspected, further evaluation may be warranted.

4-Methoxyestrone (4-M-E1)

Elevated: Methyl metabolites are considered inactive and are correlated with protective and antiproliferative effects. Proper elimination of 4-M-E1 requires optimal excretion via the GI tract; optimizing GI health is an option. To fully understand this value, it may be beneficial to examine the 4-M-E1 / 4-OH-E1 ratio.

Low (males): Lower levels of 4-M-E1 are associated with a higher risk of certain cancers and other markers for breast health in females. Due to limited research in this area, it can be postulated that males may carry similar risk. Low levels of 4-M-E1 may indicate the possibility that 4-OH metabolites are favoring the quinone/semi quinone pathway which can lead to DNA damage. Increased support of the COMT enzyme (methylation) may be an option. To fully understand this value, it may be beneficial to examine the 4-M-E1 / 4-OH-E1 ratio.

Low (females): Lower levels of 4-ME1 are associated with a higher risk of certain cancers and other markers for breast health. Low levels of 4-M-E1 may indicate the possibility that 4-OH metabolites are favoring the quinone/semi quinone pathway which can lead to DNA damage. Increased support of the COMT enzyme (methylation) may be an option. To fully understand this value, it may be beneficial to examine the 4-M-E1 / 4-OH-E1 ratio.

Estradiol (E2)

Elevated (males): Estradiol level is most consistent with exogenous exposure, supplementation, or aromatization of testosterone to estradiol. CYP19, also known as aromatase, can be upregulated raising intracellular estrogens in men which can contribute to increased adiposity, metabolic syndrome, and prostate pathology. CYP19 enzyme is induced during times of stress, exposure to xeno-estrogens, high glycemic diet, excessive adipose tissue, and alcohol consumption.

Elevated (females): Elevated estradiol level may be due to exogenous hormone supplementation or aromatization from testosterone in peripheral tissues. CYP19 enzyme, also known as aromatase, is induced during times of stress, exposure to xeno-estrogens, high glycemic diet, excessive adipose tissue, and alcohol consumption.

Low (males): Low estradiol levels may reflect deficient hormone production or excessive hormone metabolism. Confirmation of low endogenous hormone levels via saliva or serum may be warranted.

Low (females): Low estradiol levels may reflect deficient hormone production, suppressed ovarian function (anovulation) or excessive hormone metabolism. Confirmation of low endogenous levels via saliva or serum may be warranted.

2-Hydroxyestradiol (2-OH-E2)

Elevated (males): Adequate levels of 2-OH-E2 have been shown to be a marker for breast health in women, and while research in males in this area is lacking, the health benefits of this metabolite may be similar for males. Elevated levels can be due to high endogenous estradiol as well as exogenous exposure or supplementation of estrone and/or estradiol. While 2-OH-E2 is considered the "safer" estrogen metabolite, optimizing its methylation and excretion via the COMT enzyme may be beneficial.

Elevated (females): Adequate levels of 2-OH-E2 have been shown to be a marker for breast health. Elevated levels can be due to high endogenous estradiol as well as exogenous exposure or supplementation of estrone and/or estradiol. While 2-OH-E2 is considered the "safer" estrogen metabolite, optimizing its methylation and excretion via the COMT enzyme may be beneficial.

Low (males): Adequate levels of 2-OH-E2, the "safer" estrogen metabolite, have been shown to be a marker for breast health in females. While research in males is lacking, it is possible that men may have similar protection. Low levels of 2-OH-E2 may be due to low levels of estradiol, estrone, or more active CYP34A or CYP1B1 enzymes. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

Low (females): Adequate levels of 2-OH-E2, the "safer" estrogen metabolite, have been shown to be a marker for breast health. Low levels of 2-OH-E2 may be due to low levels of estradiol, estrone, or more active CYP34A or CYP1B1 enzymes. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

4-Hydroxyestradiol (4-OH-E2)

Elevated (males): Elevated levels are associated with higher risk of certain cancers and other negative markers of breast health in females. Due to the lack of research in this area, it may be postulated that males carry a similar risk. Elevation of 4-OH-E2 may be due to an overactive CYP1B1 enzyme or sluggish CYP1A1 or CYP3A4. Research indicates CYP1B1 is upregulated by PAHs, PCBs, THC, UV exposure, leptin resistance, inflammation, and insulin resistance. Supporting the COMT enzyme (methylation) is a consideration.

Elevated (females): Elevated levels are associated with higher risk of certain cancers and other negative markers of breast health in females. Elevation of 4-OH-E2 may be due to an overactive CYP1B1 enzyme or sluggish CYP1A1 or CYP3A4. Research indicates CYP1B1 is upregulated by PAHs, PCBs, THC, UV exposure, leptin resistance, inflammation, and insulin resistance. Supporting the COMT enzyme (methylation) is a consideration.

Low (males): 4-OH-E2 is associated with a higher risk of certain cancers and other negative markers for breast health in females. Due to the lack of research in this area, it may be postulated that males carry a similar risk. Low levels may be beneficial depending on the other metabolites and methylation further down the pathway.

Low (females): 4-OH-E2 is associated with a higher risk of certain cancers and other negative markers for breast health in women. Low levels may be beneficial depending on the other metabolites and methylation further down the pathway.

2-Methoxyestradiol (2-M-E2)

Elevated: 2-M-E2 is considered non-reactive and protective. Higher levels have been correlated with antiproliferative, antiangiogenic, and cardioprotective properties. Depending on other metabolite values and a proper function of the GI tract, elevations of 2-M-E2 may be considered healthy.

Low (males): 2-M-E2 is considered a non-reactive metabolite. Lower levels have been associated with a higher risk of certain cancers and other negative markers of breast health in women. Due to the lack of research, it may be postulated that males carry a similar risk. Supporting the COMT enzyme (methylation) is a consideration.

Low (females): 2-M-E2 is considered a non-reactive metabolite. Lower levels have been associated with a higher risk of certain cancers and other negative markers of breast health in females. Supporting the COMT enzyme (methylation) is a consideration.

4-Methoxyestradiol (4-M-E2)

Elevated: Methyl metabolites are considered inactive and are correlated with antiproliferative effects. Proper elimination of 4-M-E2 requires optimal excretion via GI tract optimization. To fully understand this value, it may be beneficial to examine the 4-M-E2 / 4-OH-E2 ratio.

Low (males): Lower levels of 4-M-E2 is associated with a higher risk of certain cancers and other negative markers for breast health in females. Due to lack of research in this area, it can be postulated that males

carry a similar risk. Low levels of 4-M-E2 may indicate that 4-OH metabolites are favoring the quinone/semi quinone pathway which can lead to DNA damage. Supporting the COMT enzyme (methylation) is a consideration.

Low (females): Lower levels of 4-M-E2 is associated with a higher risk of certain cancers and other negative markers for breast health. Low levels of 4-M-E2 may indicate that 4-OH metabolites are favoring the quinone/semi quinone pathway which can lead to DNA damage. Supporting the COMT enzyme (methylation) is a consideration.

Estriol (E3)

Elevated: Estriol is above the reference range which is likely due to individual variance, supplementation, or exogenous exposure. Increased metabolism from 16-OH-E1 via HSD17β may also be a contributing factor. Estriol is considered a safer estrogen due to its inability to convert back to estrone or estradiol. Elevations may have little clinical significance if other metabolite levels seem appropriate.

Low (males): The low estriol level may be due to decreased conversion from estrone, estradiol and/or 16-OH-E1.

Low (females): The low estriol level may be due to decreased conversion from estrone, estradiol and/or 16-OH-E1. In females, lower estriol levels may be associated with vaginal dryness. Supplementation with estriol is a consideration.

Ratios and Calculations

Percentages of 2-OH-E1, 4-OH-E1, and 16-OH-E1

When evaluating phase I metabolism, it can be helpful to compare the percentages of 2, 4, and 16 OH-E1 metabolites. Most individuals metabolize the majority of their estrogens down the 2-OH-E1 pathway which is generally considered the "safer pathway". This is followed by 16-OH-E1 and 4-OH-E1 respectively, both of which are deemed more reactive and potentially genotoxic.

2-M-E1:2-OH-E1 (COMT/Methylation activity)

Elevated: The relationship of 2-M-E1 / 2-OH-E1 represents the activity of COMT (methylation). While 2-OH-E1 is considered a safe metabolite, it is still considered a reactive metabolite until methylated and inactivated. Elevated COMT activity shows more of 2-OH-E1 is being methylated, which is considered favorable. Over time, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 4-M-E1/ 4-OH-E1) may give more insight into the function of this enzyme.

Low: The relationship of 2-M-E1 / 2-OH-E1 represents the activity of COMT (methylation) enzyme. A low ratio indicates slower COMT activity. While 2-OH-E1 is considered a safe metabolite, it is still considered a reactive metabolite until methylated and inactivated. Comparing additional areas of COMT activity (ie 4-M-E1/ 4-OH-E1) may give more insight into the function of COMT.

2-M-E2:2-OH-E2 (COMT/Methylation activity)

Elevated: The relationship of 2-M-E2 / 2-OH-E2 represents the activity of COMT (methylation). While 2-OH-E2 is considered a safe metabolite, it is still considered a reactive metabolite until methylated and inactivated. Elevated COMT activity shows more of 2-OH-E2 is being methylated, which is considered favorable. Over time, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 4-M-E1/ 4-OH-E1) may give more insight into the function of this enzyme.

Low: The relationship of 2-M-E2 / 2-OH-E2 represents the activity of COMT (methylation) enzyme. A low ratio indicates slower COMT activity. While 2-OH-E2 is considered a safe metabolite, it is still considered a reactive metabolite until methylated and inactivated. Comparing additional areas of COMT activity (ie 4-M-E1/ 4-OH-E1) may give more insight into the function of this enzyme.

4-M-E1:4-OH-E1 (COMT/Methylation activity)

Elevated: The relationship of 4-M-E1 / 4-OH-E1 represents the activity of COMT (methylation). 4-OH-E1 is considered unfavorable due to its carcinogenic potential within breast and prostatic tissue. Elevated COMT activity shows more of 4-OH-E1 is being methylated, which is considered favorable. Overtime, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 2-M-E1/ 2-OH-E1) may give more insight into the function of this enzyme.

Low: The relationship of 4-M-E1 / 4-OH-E1 represents the activity of COMT (methylation) enzyme. A low ratio indicates slower COMT activity, which may mean a higher potential for the creation of quinones, semi-quinones, and depurinating adducts. Increasing COMT enzyme activity is a consideration.

4-M-E2:4-OH-E2 (COMT/Methylation activity)

Elevated (males): The relationship of 4-M-E2 / 4-OH-E2 represents the activity of COMT (methylation). 4-OH-E2 is considered unfavorable due to its carcinogenic potential within breast and prostatic tissue. Elevated COMT activity shows more of 4-OH-E1 is being methylated, which is considered favorable. Overtime, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 2-M-E1/ 2-OH-E1) may give more insight into the function of this enzyme.

Elevated (females): The relationship of 4-M-E2 / 4-OH-E2 represents the activity of COMT (methylation). 4-OH-E2 is considered unfavorable due to its carcinogenic potential within breast tissue. Elevated COMT activity shows more of 4-OH-E1 is being methylated, which is considered favorable. Overtime, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 2-M-E1/ 2-OH-E1) may give more insight into the function of this enzyme.

Low: The relationship of 4-M-E2 / 4-OH-E2 represents the activity of COMT (methylation) enzyme. A low ratio indicates slower COMT activity, which may mean a higher potential for the creation of quinones, semi-quinones, and depurinating adducts. Increasing COMT enzyme activity is a consideration.

2-OH-E1:16-OH-E1

Elevated (males): 2-OH-E1 has been shown to be a favorable marker for breast health in females due to its anti-cancer properties. Because 2-OH-E1 is considered "less estrogenic" than the more potent 16-OH-E1 pathway, a higher ratio is considered favorable for breast health. While research in men in this area is lacking, the health benefits may be similar.

Elevated (females): 2-OH-E1 has been shown to be a favorable marker for breast health in females due to its anti-cancer properties. Because 2-OH-E1 is considered "less estrogenic" than the more potent 16-OH-E1 pathway, a higher ratio is considered favorable for breast health.

Low (males): 16-OH-E1 has been shown to be more estrogenic than 2-OH-E1 with properties similar to estrone. A lower ratio favors the 16-OH-E1 pathway and could indicate increased carcinogenic potential. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

Low (females): 16-OH E1 has been shown to be more estrogenic than 2-OH-E1 with properties similar to estrone. A lower ratio favors the 16-OH-E1 pathway and could indicate an increased potential for breast cancer. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

4-OH-E1:2-OH-E1

Elevated (males): 4-OH-E1 is considered unfavorable due to its association with breast and prostate cancer. 2-OH-E1 has been shown to be a favorable marker for breast health in females due to its anti-cancer properties. A higher ratio of 4-OH-E1/2-OH-E1 has the potential to be more carcinogenic/genotoxic. Optimizing methylation to support the COMT enzyme can potentiate the more protective 2-OH-E1 pathway. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

Elevated (females): 4-OH-E1 is considered unfavorable due to its association with breast cancer and uterine fibroids. 2-OH-E1 has been shown to be a favorable marker for breast health in females due to its anti-cancer properties. A higher ratio of 4-OH-E1/2-OH-E1 has the potential to be more carcinogenic/genotoxic. Optimizing methylation to support the COMT enzyme can potentiate the more protective 2-OH-E1 pathway. Increasing the activity of CYP1A1 to increase 2-OH-E1 is a consideration.

Low (males): A low ratio can indicate a metabolic preference for the more favorable 2-OHE1 pathway, which research indicates is less genotoxic/more protective to breast and prostate cells.

Low (females): A low ratio can indicate a metabolic preference for the more favorable 2-OH-E1 pathway, which research indicates is less genotoxic/more protective to breast cells.

Key Relationships

5-α reductase activity AN:ET

Elevated (males): This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Elevated levels may indicate higher 5-alpha reductase activity which may lead to an increase in androgenic symptoms.

Elevated (females): This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Elevated levels may indicate higher 5-alpha reductase activity which may lead to an increase in androgenic symptoms like hirsutism and scalp hair loss.

Low: This ratio can be used to evaluate the presence or absence of 5-alpha reductase upregulation, down regulation, or inhibition. Decreased levels may be due to the use of 5-alpha reductase inhibition medication or inherent preference for 5-beta metabolism.

Aromatase Activity for A4:E1 and T:E2

Elevated (males): Aromatase (CYP19) is an enzyme that converts testosterone to estradiol and androstenedione to estrone. The activity of this enzyme, concentrated in peripheral adipose tissue, is increased due to inflammation, insulin resistance, and obesity. Evaluating the clinical utility of this enzyme may require understanding of estrogen metabolism as well.

Elevated (females): Aromatase (CYP19) is an enzyme that converts testosterone to estradiol and androstenedione to estrone. The activity of this enzyme, concentrated in peripheral adipose tissue, is increased due to inflammation, insulin resistance, and obesity. In post-menopausal women, aromatase may be slightly elevated as this is a major source of estrogen production in this population, often leading to elevations in estrogen levels. Evaluating the clinical utility of this enzyme may require an understanding of estrogen metabolism as well.

Low (males): Aromatase (CYP19) is an enzyme that converts testosterone to estradiol and androstenedione to estrone. Low activity of this enzyme may be beneficial as it may preserve free testosterone.

Low (females): Aromatase (CYP19) is an enzyme that converts testosterone to estradiol and androstenedione to estrone. Research on low rates of aromatase activity in females is limited. Understanding the full clinical implications of this enzyme may require further investigation of estrogen metabolism.

COMT Activity 4-M-E1:4-OH-E1

Elevated: The relationship of 4-M-E1 / 4-OH-E1 represents the activity of COMT (methylation). 4-OH-E1 is considered unfavorable due to its carcinogenic potential within breast and prostatic tissue. Elevated COMT activity shows more of 4-OH-E1 is being methylated, which is considered favorable. Overtime, COMT enzyme may need additional support to keep up with demand. Comparing additional areas of COMT activity (ie 2-M-E1/ 2-OH-E1) may give more insight into the function of this enzyme.

Low: The relationship of 4-M-E1 / 4-OH-E1 represents the activity of COMT (methylation) enzyme. A low ratio indicates slower COMT activity, which may mean a higher potential for the creation of quinones, semi-quinones, and depurinating adducts. Increasing COMT enzyme activity is a consideration.

Additional Factors Affecting Metabolism

Thyroid

Thyroid hormones are in control of the body's metabolism including steroid hormones. Therefore, the health of the thyroid gland also has the potential to affect the amount of steroid hormones as well as their metabolites. Generally, hyperthyroidism will relate to increased metabolism while hypothyroid states will lead to a slower metabolism.

To determine how an individual's thyroid status might be affecting urinary hormone and metabolites, there are a few areas of the HuMap™ that might give some clues.

Progesterones

Hypothyroidism can lead to anovulation which will result in low progesterone levels, and subsequently the metabolites of progesterone.

Testosterone

Sex Hormone Binding Globulin (SHBG) is the major sex hormone carrier protein in serum. Under physiological conditions, approximately 70% of testosterone is bound to SHBG with high affinity, about 20–30% is weakly bound to albumin, and the remaining 1-2% is free.

Thyroid hormones increase SHBG production indirectly by increasing HNF-4alpha gene expression, and by reducing cellular palmitate levels that further contribute to increased HNF-4alpha levels in hepatocytes. Human SHBG binds dihydrotestosterone (DHT) > testosterone > estradiol.

SHBG transports testosterone and other steroids in the blood plasma, reduces their metabolic clearance rate, and regulates their access to target tissues. Hypothyroidism may result in lower SHBG and less testosterone metabolic clearance.

Corticoids

Urinary cortisol metabolites are altered both quantitatively and qualitatively in thyroid dysfunction. In hyperthyroidism the rate of cortisol clearance tends to be higher as well as metabolized cortisol. In hypothyroidism, the rate of cortisol clearance slows and there may be lower levels of metabolized cortisol.

Estrogens

Research has shown that hypothyroidism is associated with a reversible partial suppression of the HPGA (hypothalamo-pituitary-gonadal axis) in premenopausal women resulting in lower E2 and mild elevation of prolactin. The evidence suggest treatment of hypothyroidism improves the level of estrogen and lowers the level of prolactin.

Other studies have found that elevated TSH correlated with lower serum E2 and T, which was normalized when euthyroid status (normal TSH) was reached. It has been suggested that urinary estradiol is relatively consistent to serum estradiol. This might indicate that a hypothyroid status could result in lower-than-normal urinary metabolites of E2 and T.

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OCPs

Oral contraceptives (OCPs), are used in a variety of patients and for more than just contraceptive reasons. Whether the target of this therapy is for pregnancy prevention or acne, the mechanism of action remains the same: suppression of FSH and LH to decrease estrogen and prevent ovulation which also leads to a decrease in the production of progesterone. With suppression of estrogen and progesterone production, lower levels of their metabolites may also be expected.

Interestingly, cortisol metabolism may also be affected by OCP use. Several studies suggest OCPs can increase circulating CBG (cortisol binding globulin) leading to a decrease in total cortisol concentrations as well as metabolites. Progestin-only and low-dose estrogen contraceptives have been shown to have less effect on cortisol levels in plasma and saliva, but this has not been studied in urine.

There is no current research advising how best to time collection for urinary hormone and metabolite testing in patients on OCPs. Collecting during days 19-23 of a cycle (what would be the luteal surge) is likely a good rule of thumb, although collecting any time may be appropriate.

It is not recommended that women stop OCPs for the purposes of testing. Because the mechanism of the pill is to suppress ovulation, and OCPs can increase binding proteins like CBG, results will reflect the influence of the OCPs which can be seen in estrogens, progesterones, and cortisol levels. If assessing endogenous levels is desired, she will need to discontinue OCPs for a full 3 months before testing.

Hormonal contraceptives other than OCPs can also influence hormone levels. Progestin implants and shots (i.e. Nexplanon or Depo Provera) also suppress ovulation, and therefore estrogen and progesterone levels will be affected. However, these methods are less likely to affect cortisol levels. Progestin IUDs, like the Mirena, also will not affect cortisol levels. This method is also less likely to affect estrogens and progesterones.

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Estrobolome

Gut bacteria have numerous physiologic implications. Within the colon, specific bacteria play a role in estrogen metabolism. This microbiome made up of colonic bacteria that induce estrogen metabolism is termed the "estrobolome." Robust microbial abundance and diversity is associated with proper estrogen metabolism via the estrobolome. Conversely, gut microbial dysbiosis is associated with impaired estrogen metabolism. Additional factors influencing the estrobolome include genetics, diet, sugar, alcohol, medications and environmental exposures.

Estrogen is both endogenous and exogenous. Endogenous estrogen includes three forms; the post-menopausal dominant estrone (E1), the major form estradiol (E2), and least potent estriol (E3). Exogenous estrogen may be derived via dietary phytoestrogens and xenoestrogens. Xenoestrogens may be found in common household items, such as beauty products and plastics. Regardless of the source, all estrogens must be metabolized.

Once in the intestines, estrogen is either metabolized and eliminated, or reabsorbed and recirculated. Conjugation is necessary for estrogen to be metabolized. An estrobolome rich in bacterial species, such as *Bacteroides* and *Clostridia*, produces the optimal amount of beta-glucuronidase to conjugate estrogen delivering it to the bile for excretion into the gut. A healthy estrobolome minimizes reabsorption of estrogen from the gut, enabling hormone excretion in stool and urine, which may support hormonal balance.

Beta-glucuronidase is an enzyme that breaks the tight bonds between glucuronic acid and estrogen in the colon. Glucuronidation via beta-glucuronidase provides a major route of detoxification and estrogen metabolism. Anaerobic bacteria such as *Bacteroides* and *Clostridia* produce beta-glucuronidase. In this manner, colonic bacteria play a role in estrogen metabolism. Higher levels of beta-glucuronidase may be associated with higher circulating estrogens and lower fecal excretion of estrogens in premenopausal women. Gut dysbiosis can produce an excess of beta-glucuronidase, which can lead to the deconjugation of estrogen, reverting it back to its active form which is then absorbed into the bloodstream contributing to hormonal imbalance.

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Methylation

Methylation is an important factor in healthy metabolism as the disruption of normal methylation patterns has been found to lead to carcinogenesis. In the case of estrogen metabolism, hydroxy estrogens (-OH) are methylated to methoxy estrogens (-M). This process is considered protective as methylation of hydroxy estrogens marks them for elimination from the body. Without this process, further conversion of hydroxy estrogens could lead to even more reactive quinone estrogens. Evaluation of proper methylation within the estrogen pathway can be examined by assessing the ratio of hydroxy estrogens (2-OH E1/E2 and 4-OH E1/E2) to methoxy estrogens (2-M E1/E2 and 4-M E1/E2). If results reveal low levels of unconjugated estrogen (E1 and E2) to be low, one might expect the levels of methylated estrogens to be low. However, if hydroxylated estrogens levels are elevated and methylated estrogen levels are low, this could indicate inefficient methylation. Additionally, deficiency in methylation could potentially result from genetic variants in COMT which could decrease enzyme activity.

COMT aids in the break down of catecholamines as well as hydroxy estrogens. Alteration in the COMT gene can lead to a build up of hydroxy-estrogens. COMT utilizes SAMe as its methyl donor and magnesium as its nutrient cofactor. Estrogen's genotoxic potential varies across individuals and may be influenced by genetic variation within the hydroxy estrogen pathway.

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DOCTOR'S DATA INC

SCIENCE + INSIGHT

3755 Illinois Avenue • St. Charles, IL 60174-2420

800.323.2784 (US AND CANADA)
+1.630.377.8139 (GLOBAL)

doctorsdata.com