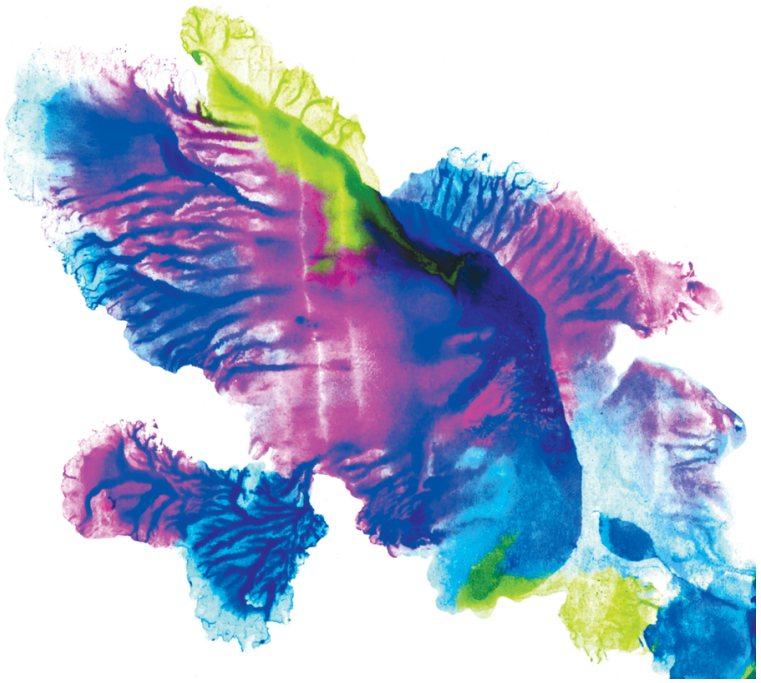
Comprehensive Hormone Insights™

COMPREHENSIVE HORMONE INSIGHTS™
SAMPLE TEST REPORT

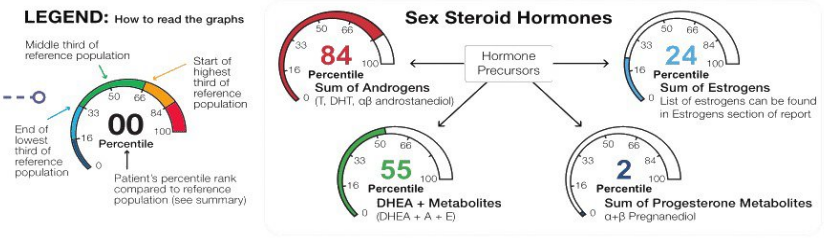


Cycling Female
Dr. Who
Rocky Mountain Analytical

How to read a CHI report

SUMMARY DIALS

The first page of the report summarizes key hormone measures including: androgens, estrogens, progesterone metabolites, DHEA & metabolites, 8OH2dG, 6-sulfatoxymelatonin and the ratio of cortisol to testosterone.

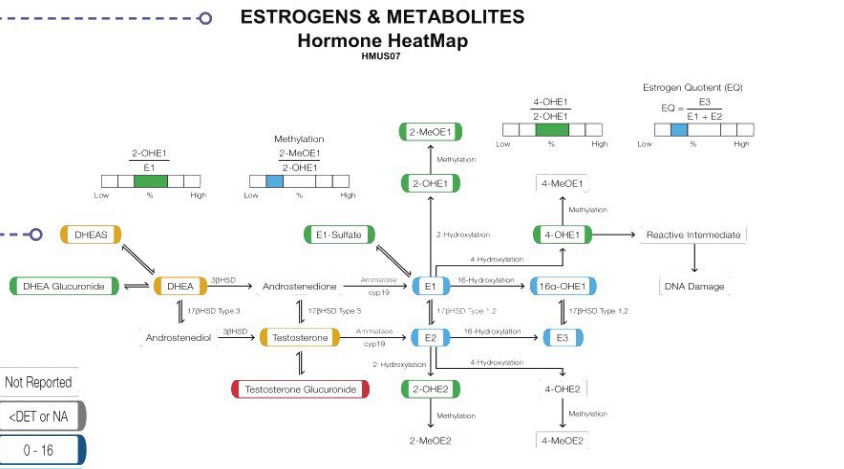


HORMONE CATEGORY

Each hormone category has its own heatmap and corresponding bar graphs for individual analytes.

HEATMAP

Hormone endcap colour immediately identifies which hormone levels are high, high normal, normal, low normal and low.



LEGEND

Identifies by colour what the patient percentile "zone" is for each reported analyte or ratio.

RESULTS & RANGES

This section reports the results, ranges and graphs for the percentiles of individual analytes and sums/ratios. Range applied is specific to patient: male, female and whether luteal, follicular or post-menopausal.

Reference range low is 20th percentile, and high is 80th percentile.

ESTROGENS

Analyte	Result	Range	Units	0% 16% 33% 66% 84% 100%	%Ile	Range Applied
Estrone (E1)	6.7	5.3 - 15	ng/mL		31%	d21
Estradiol (E2)	2.4	2.3 - 5.5	ng/mL		25%	d21
Estriol (E3)	7.2	6.7 - 39	ng/mL		24%	d21
E3/(E1 + E2)	0.79	0.65 - 2.7			26%	d21
2-Hydroxyestrone (2-OHE1)	6.0	3.0 - 12	ng/mL		41%	d21
2-OHE1/E1	0.89	0.38 - 0.99	ng/mL		66%	d21
2-Methoxyestrone (2-MeOE1)	1.5	0.79 - 4.5	ng/mL		39%	d21
2-MeOE1/2-OHE1	0.25	0.21 - 0.55			29%	d21
16a-Hydroxyestrone (16a-OHE1)	2.7	2.3 - 10	ng/mL		25%	d21
2-OHE1/16a-OHE1	2.2	0.43 - 2.6			69%	d21
4-Hydroxyestrone (4-OHE1)	0.98	0.53 - 2.1	ng/mL		41%	d21
4-OHE1/2-OHE1	0.16	0.13 - 0.26			40%	d21
2-Hydroxyestradiol (2-OHE2)	0.94	0.42 - 2.8	ng/mL		48%	d21
2-Methoxyestradiol (2-MeOE2)	< 0.30	0.085 - 4.1	ng/mL		<DET	d21
Estrone sulphate (E1-Sulphate or E1S)	0.95	0.45 - 2.0	ng/mL		55%	d21
Sum of Estrogens	28	27 - 87	ng/mL		24%	d21

COMMENTARY

Custom interpretation comments appear after each hormone category.

INTERPRETATION WHEN MULTIPLE ESTROGENS AND ESTROGEN METABOLITES ARE LOW
Many estrogenic hormones and hormone metabolites measured here are low. This pattern may still be quite clinically meaningful; however, prudence should be used when attempting to correlate and compare results via ratios. The clinician has to always bear in mind that the ratio may be less significant when the results making up the numerator and denominator are low compared to ratios constructed from results that are normal or elevated.

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Rocky Mountain Analytical®
Changing lives, one test at a time

Date of Collection: 2020/02/21
Time of Collection: 9:30AM
Date of Receipt:
Reported On:

CHI SAMPLE REPORT

Accession

Healthcare Professional

Rocky Mountain Analytical
Dr. Who
105 - 32 Royal Vista Drive NW
Calgary, AB T3R0H9

P: 403-241-4500
F: 403-241-4501

Patient

Cycling Female

Age: 24
Date of Birth:
Gender: Female
Status: Regular
Cycle Day: 20

Relevant Medications	Biometrics
	Height (in) : 63
	Weight (lb) : 125
	BMI : 22
	Waist (in) : 0
	Hip (in) : 0

CHI

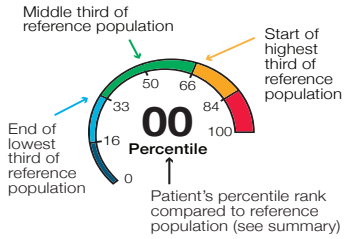
Accession

Comprehensive Hormone Insights™

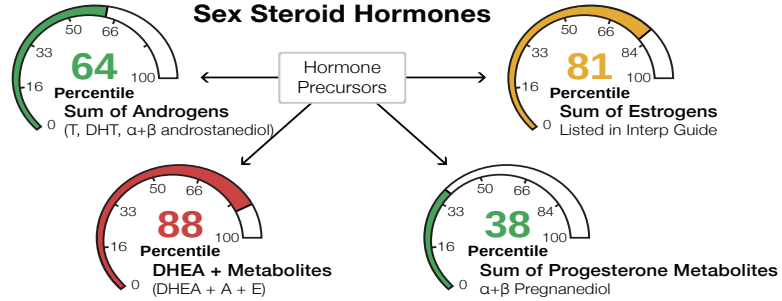
SUMMARY

HMUS01

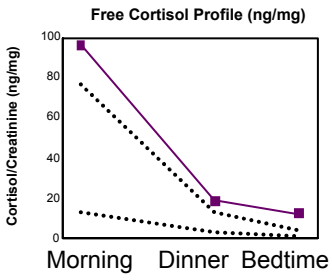
LEGEND: How to read the graphs



Sex Steroid Hormones

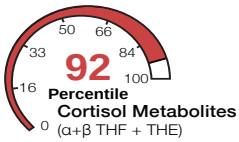


Cortisol

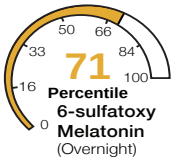


Free cortisol profile is used to assess diurnal cortisol rhythm

Cortisol Metabolites provides a general assessment of adrenal cortisol production

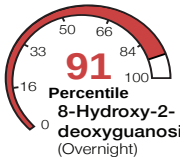


Melatonin

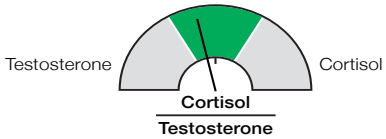


6-sulfatoxymelatonin provides insight into melatonin levels.

Oxidative Stress



8-hydroxy-2-deoxyguanosine is a marker of oxidative stress



Cortisol/Testosterone provides insight into relative catabolic (cortisol) and anabolic (testosterone) states.

RATIONALE FOR APPLICATION OF LUTEAL REFERENCE RANGES

Based on the information provided, the patient is in the luteal phase of her cycle. For cycles that are routinely less than 28 days long, the luteal phase is considered to start 14 days before the next bleeding. So for example, in a 21 day cycle, the luteal phase would start on Day 7, for a 25 day cycle it would start on Day 11, and so forth.

SYMPTOM INVENTORY WAS LEFT BLANK

The symptom inventory was left blank. In some cases, this may limit the commentary for various of the test results. Collection of symptom information assists in the refinement of future interpretations, as it allows the discovery of correlations between symptoms and metabolite levels.

8-HYDROXY-2-DEOXYGUANOSINE RESULT IS ELEVATED

Oxidative stress is a term that describes cellular damage from reactive oxygen free radicals (ROS) and reactive nitrogen species (RNS). When ROS react with DNA, the DNA repair process causes 8-hydroxy-2-deoxyguanosine (8OH2dG) to be released and excreted in urine. Measurement of urinary 8OH2dG is therefore a considered a biomarker of DNA damage and oxidative stress.

Free radical formation is a normal part of aerobic metabolism, and some ROS are needed for proper cellular function. Free radicals form naturally with inflammation and cellular metabolism, but can be increased by environmental factors such as excessive UV light, air pollution, ionizing radiation and smoking. Anti-oxidant molecules found in the body and in food can bind to and neutralize free radicals. However, when free radicals outnumber anti-oxidants, damage to lipid membranes, proteins, and DNA can follow.

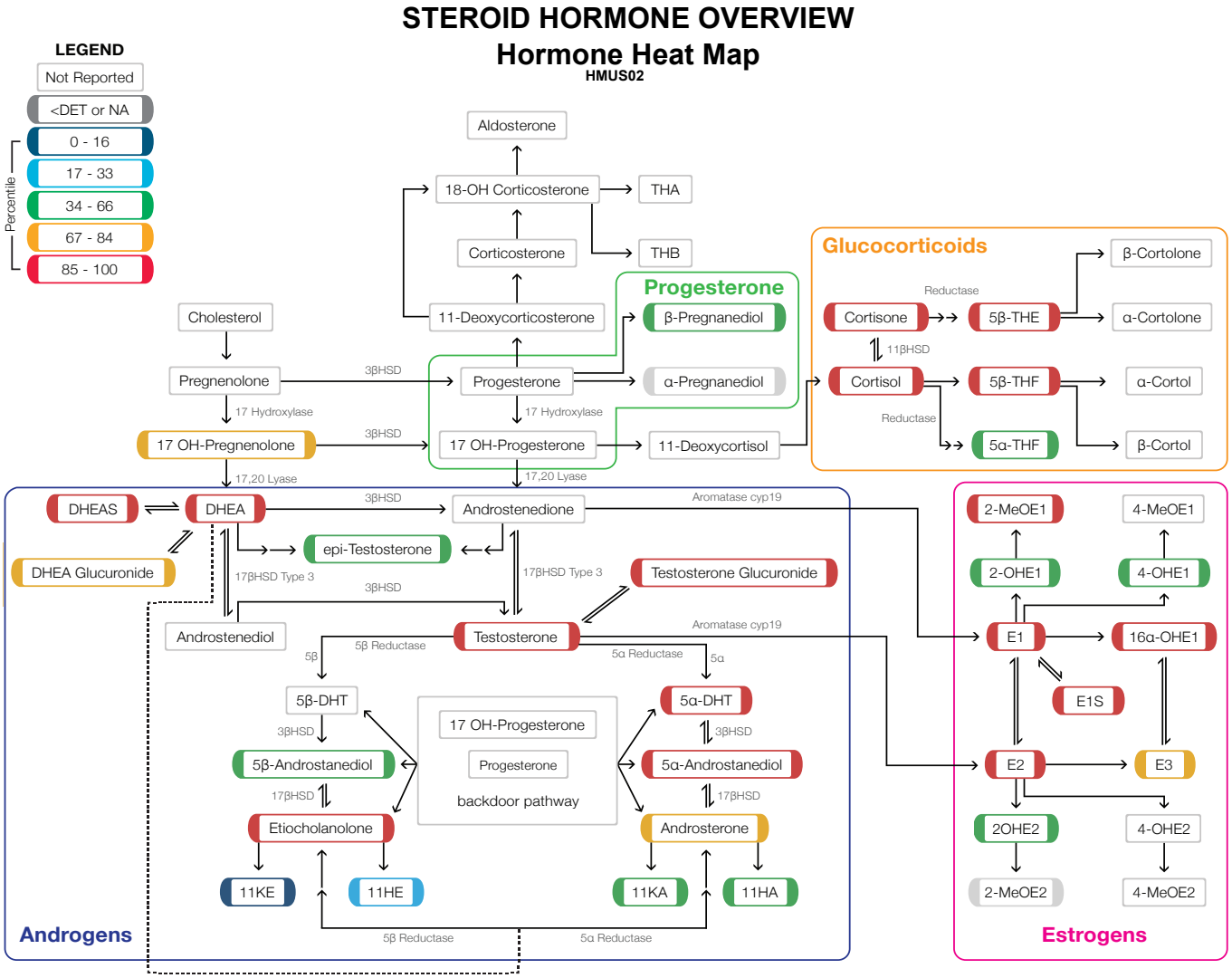
Oxidative stress has been implicated in a number of disease states: cardiovascular disease (references: Bonomini 2008, Sinha 2015, Touyz 2011), cancers (Halliwell 2007), depression and Parkinson's disease (Blesa 2015), chronic fatigue (Kennedy 2005). Excessive oxidative stress may also result in a general impairment of mitochondrial steroidogenesis..

The result for 8-hydroxy-2-deoxyguanosine lies above the 66th percentile.

Consideration needs to be given to reducing exposure to offending influences, implementing detoxification strategies and supplementation with antioxidants and precursors supportive of glutathione synthesis (NAC, sulfur-containing foods).

MARKERS REFLECTING THYROID HORMONE ACTIVITY

There are multiple ratios and patterns measured in this test that can potentially inform on the overall activity of thyroid hormones. The interpretation is "tuned" to comment on thyroid hormone activity only when there is sufficient alignment amongst all the various indicators. If alignment is not sufficient, various individual comments about thyroid hormone activity may appear but there will be no overall "verdict" regarding thyroid hormone activity. When multiple indicators are measured simultaneously, chance variations can decrease the likelihood that all of the indicators will align perfectly, even in the face of a bona fide thyroid hormone issue.



The Rocky Mountain Analytical Hormone HeatMap provides an overview of sex steroid hormone and hormone metabolite results. For each of the Hormone HeatMap pathways, the colour that frames the named hormone or metabolite corresponds to the percentile found in the Legend (top left). This makes deficiencies or excesses in the major hormone groups easy to identify and patterns easier to discern.

Note: Hormones bordered in gray are either not tested or not reported. They are included for completeness.

HORMONE ABBREVIATIONS

5ß-THE: 5ß-tetrahydrocortisone
5α-THF: 5α-tetrahydrocortisol
5ß-THF: 5ß-tetrahydrocortisol
11HA: 11-hydroxyandrosterone
11HE: 11-hydroxyetiocholanolone
11KA: 11-ketoandrosterone
11KE: 11-ketoetiocholanolone
DHEAS: dehydroepiandrosterone sulfate
DHEA: dehydroepiandrosterone

E1: estrone
E2: estradiol
E3: estriol
2-MeOE1: 2- methoxyestrone
2-MeOE2: 2-methoxyestradiol
2-OHE1: 2-hydroxyestrone
4OHE1: 4-hydroxyestrone

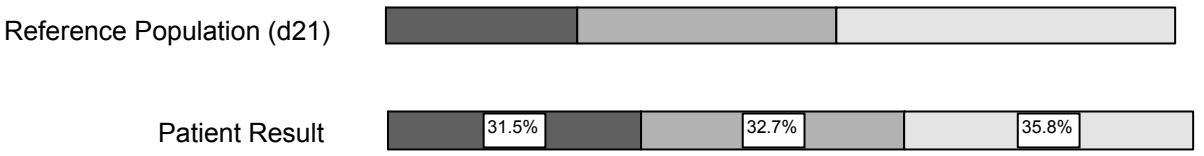
2OHE2: 2-hydroxyestradiol
4OHE2: 4-hydroxyestradiol
16α-OHE1: 16α-hydroxyestrone

CORTISOL & CORTISOL METABOLITES Hormone Heat Map

HMUS03

CORTISOL CONJUGATION PATTERN

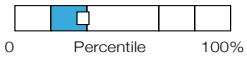
Cortisol (free)	Cortisol sulfate	Cortisol glucuronide
15.6 - 34 %	25.3 - 36.6 %	36.6 - 51.3 %



Cortisol-Cortisone Metabolite Balance

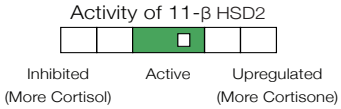
Cortisol Clearance Ratio

The ratio (αTHF+ βTHF + βTHE) / (Cortisol + Cortisone) provides insight into the overall "speed" of reduction or hydrogenation of cortisol and cortisone.



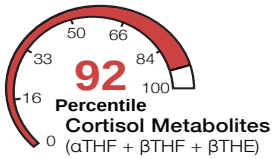
Activity of 11-β HSD2

This marker informs on the nature of the activity of 11-β HSD2 enzyme. 11-β HSD2 converts cortisol to inactive cortisone in kidneys, distal colon and saliva glands. When this enzyme is markedly inhibited free cortisol will be much greater than free cortisone. When the enzyme is upregulated free cortisone will typically be much greater than free cortisol.

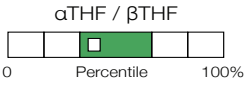


Cortisol Metabolites

Insight into adrenal production of cortisol metabolites.



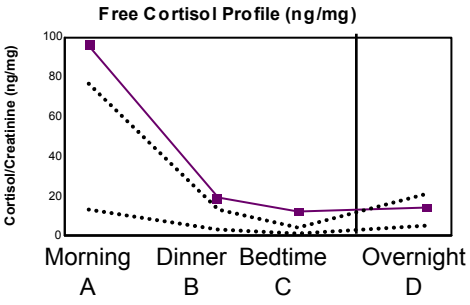
Alpha Reductase Preference



Alpha reductase preference may be influenced by thyroid status.

Free Cortisol Profile

The dotted lines represent the 20th and 80th percentiles for each cortisol point.



The Free Cortisol Profile provides insight into the diurnal rhythm of cortisol.

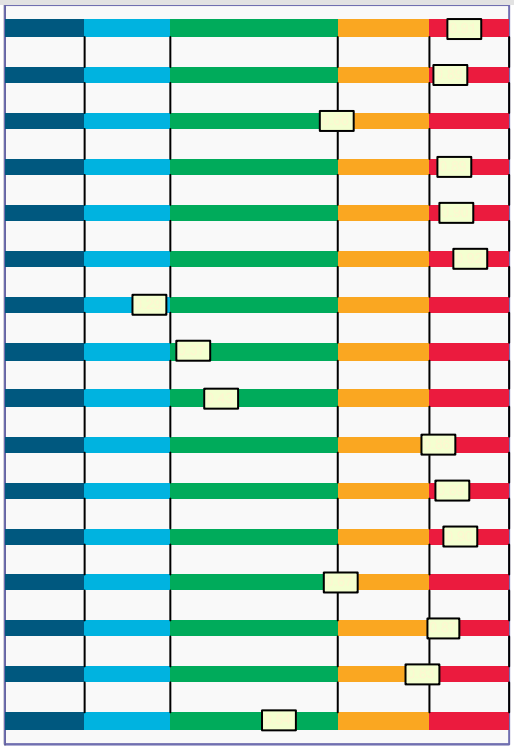
Two ratios are calculated:
- Overnight Cortisol Response (D/C)
- Morning Cortisol Response (A/D)

Neither ratio is graphed, but both are commented on in the interpretation.

CORTISOL RATIOS AND SUMS

CORTISOL & METABOLITES

Analyte	Result	Range	Units	0% 16% 33% 66% 84% 100%	%ile	Range Applied
Cortisol (GCMSMS)	130	55 - 110	ng/mg		91%	d21
Cortisone (GCMSMS)	190	86 - 140	ng/mg		89%	d21
a-Tetrahydrocortisol (aTHF)	230	100 - 330	ng/mg		66%	d21
ß-Tetrahydrocortisol (ßTHF)	1,700	870 - 1,500	ng/mg		89%	d21
ß-Tetrahydrocortisone (ßTHE)	2,800	1,500 - 2,500	ng/mg		90%	d21
aTHF+ßTHF+ßTHE	4,700	2,800 - 4,300	ng/mg		92%	d21
Cortisol Clearance Ratio	14	12 - 24			29%	d21
a-THF/ß-THF	0.14	0.095 - 0.23			37%	d21
Cortisol/Testosterone	1.0	0.53 - 2.6			43%	d21
Morning Free Cortisol (Specimen A)	96	13 - 77	ng/mg		86%	d21
Dinnertime Free Cortisol (Specimen B)	19	3.3 - 14	ng/mg		89%	d21
Bedtime Free Cortisol (Specimen C)	12	1.8 - 4.5	ng/mg		90%	d21
Overnight Free Cortisol (specimen D)	14	5.3 - 21	ng/mg		67%	d21
Free Cortisol (pooled specimen)	36	10 - 30	ng/mL		87%	d21
Free Cortisone (pooled specimen)	76	35 - 70	ng/mL		83%	d21
11-beta HSD2 Activity Ratio	1.0	0.42 - 1.8				All



(aTHF + THF + THE) RESULT ELEVATED

The result for the sum: aTHF + THF + THE lies above the 66th percentile, or the result for each metabolite individually lies above the 66th percentile. Collectively, these markers are thought to make up more than half of the metabolites of cortisol and give a good measure of cortisol secretion. Note that the clinical presentation doesn't ride solely on the total amount of cortisol secreted, but on how much free cortisol is "left on the table" to talk to receptors in target tissues. Some patients with elevated cortisol metabolites may feel fine and not complain of high cortisol symptoms nor display signs of high cortisol.

Symptoms of increased activity of cortisol at the tissue receptor level may include weight gain, high blood pressure, insulin-glucose axis imbalance, irritability, sleep disturbances as well as symptoms of antagonism of other hormones, i.e. low testosterone, low progesterone, low estrogen symptoms. At the same time, bear in mind that excess cortisol can drive conversion of androgens to estrogens by upregulating aromatase. Symptoms depend heavily on how much secreted cortisol is left in the form of cortisol. If cortisol secretion is high but most of it is deactivated to cortisone and tetrahydro-cortisone, there will be less adverse impact.

Elevated glucocorticoid metabolites may be seen in a wide range of conditions of cortisol overproduction, including Cushing's Disease, adrenal hyperplasias, adrenal adenomas, pituitary tumors, obesity, acute stress, acute illness, depression and hyperthyroidism.

Note also, that elevated glucocorticoids may be a result of supplementation with adrenal extracts or herbs intended to increase the output of the adrenals. Supplementation with progesterone and pregnenolone may elevate cortisol, especially if these hormones are administered orally or sublingually.

Interventions will depend on the clinical presentation, as always.

DETERMINANTS OF FREE CORTISOL

The amount of free cortisol relative to overall cortisol production is determined by five main factors:

1. The overall extent of production of cortisol, influenced by various factors including sleep (quantity, quality, OSA), allostatic load (stress), inflammation, thyroid hormone activity, body fat burden, calorie intake.
2. The combined rates at which alpha- and beta-reductase irreversibly convert cortisol and cortisone to hydrogenated metabolites-influenced by presence or absence of biliary stasis, hepatic inflammation, thyroid hormone activity, intake of lipotrophic nutrients and foods.
3. The tendency to preserve cortisol as cortisol or shift it to cortisone-which is subject to bile acids, insulin, exercise, % body fat, supplements
4. The extent to which conjugation (Phase II) enzymes are active-which is subject to genetics, exposure to external toxins, diet, hormones and supplements taken.
5. General nutrient intake including nutrients relevant for adrenal health (e.g. Vitamin C, B Vitamins, Mg, K, adequate Na intake).

It is difficult to identify any one factor that will be dominant: thyroid support, liver support, identification and removal/mitigation of sources of inflammation, and normalization of the insulin-glucose axis all play some role.

Here the result for free cortisol (ng/mg) in the pooled specimen lies at 91 % in the reference population.

***NOTE: The percentile for the pooled free cortisol cannot be estimated by taking the average of the percentiles for each of the four point free cortisols !!

CORTISOL CLEARANCE RATIO IS LOW NORMAL OR LOW

The Cortisol Clearance Ratio (CCR) is calculated as follows: (aTHF + bTHF + bTHE)/ (Cortisol + Cortisone). The ratio assesses the overall “speed” of the irreversible reduction of cortisol and cortisone. The two drivers of reduction are bile acids, regulating beta-reduction (AKR1D1) and androgens/androgen precursors, regulating alpha-reduction (SRD5A1).

The result for this patient's ratio is 14 and lies close to or below the 33rd percentile.

Overall, alpha-reductase activity appears low when we look at the “androgenic” chain of hormones extending from 17-hydroxypregnenolone through to androsterone, and this reduced alpha- tendency also manifests with reduced clearance of cortisol though alpha-THF formation. This is often seen when overall cortisol production is low and both alpha- and beta-reduction of cortisol are throttled back to conserve cortisol.

Downregulation of both alpha- and beta-reductase can reflect low thyroid hormone activity. Beta-reductase downregulation can also reflect elevated levels of chenodeoxycholic acid (CDCA) due to biliary stasis/obstruction or hepatic inflammation. Rarely, supplementation with CDCA will suppress beta-reductase. Use of a prescription 5-alpha reductase inhibiting drug or use of a natural product which can inhibit 5-alpha reductase may also inhibit beta reductase, but one would see low 5-alpha reduced androgens if that were the case.

Note that severe elevation of bile acids, such as that seen in cirrhosis, can lead to HPA axis suppression with adrenal atrophy. This mechanism may be playing out to a lesser extent when liver handling of bile is impaired but not drastically so, and may explain some cases of low cortisol production.

Clinical correlation is required to better understand which factor or factors might be relevant here.

RATIO: aTHF/bTHF RESULT IS LOW NORMAL OR LOW
This patient's aTHF/bTHF ratio result lies close to or below the 33rd percentile. Some factor such as low thyroid hormone activity may be acting to slow down reduction with alpha reductase affected more strongly than beta reductase. Clinical correlation, looking for signs and symptoms of decreased tissue activity of T3, is required.

A relative slowing of 5-alpha reductase activity has also been noted in diabetes (Dullaart 1995) as well as insuling resistance and fatty liver. The enzyme activity may be reduced or blocked by medications intended to reduce the symptoms of an enlarged prostate, as well as by isotretinoin (Accutane). Pharmaceutical inhibition of hepatic 5-alpha reductase can lead to fatty liver (Hazlehurst 2016) since reduction is a key mechanism whereby cortisol is irreversibly inactivated in the liver. Markedly low 5-alpha reductase activity may also arise due to loss-of-function SNPs.

Other 5-alpha reductase substrates include progesterone and androstenedione. Supplementation with progesterone in very modest doses may compete with testosterone and reduce DHT formation. Epitestosterone is also known to be an inhibitor of 5-alpha reductase (Choi 2001).

Supplements including zinc, beta-sitosterol, and various medium and long chain fatty acids (GLA, coconut oil, oleic acid) may inhibit/reduce the activity of 5-alpha reductase. There are a variety of herbs/plant extracts which also do so, including saw palmetto, green tea and Reishi mushroom (Grant 2012).

Note that when the tendency or bias seen in the ratio aTHF/bTHF (low, high, average) doesn't match the tendency displayed by Androsterone/Etiocholanolone, the tendency displayed by the Androsterone/Eticholanolone ratio should be given the most weight. The aTHF/bTHF ratio can sometimes be misleading due to methodological considerations.

LOW RATIO (aTHF + bTHF)/bTHE AND FATTY LIVER
The result for the ratio (aTHF + bTHF) / bTHE lies close to or below the 33rd percentile. Westerbacka et al studied a cohort of males ranging in age from 22 to 57 years and noted that the ratio in question was significantly lower in subjects with liver fat higher than 10% on biopsy compared to those with less than 10% liver fat (Westerbacka 2003).

Consideration should be given to the possibility of hepatic steatosis depending on the clinical presentation: body habitus, alcohol intake, markers of metabolic derangement consistent with insulin resistance, Metabolic Syndrome, markers reflective of hepatic inflammation. Recognize that these findings pertained to male subjects. Female subjects may be less susceptible to nonalcoholic steatosis.

GLUCURONIDATED CORTISOL FRACTION OF TOTAL CORTISOL IS LOW
The total cortisol result (measured by GC-MSMS after sample hydrolysis) is comprised of three major fractions measured by LC-MSMS without hydrolysis: non-conjugated or free cortisol, cortisol glucuronide and cortisol sulfate.

The free cortisol and cortisol sulfate fractions are within the middle 60% of the respective reference ranges, between the 20th and 80th percentiles. The percentage of total which is cortisol glucuronide lies at or below the 33rd percentile.

When the proportion of cortisol glucuronide is markedly below the low end of its respective range this may be indicative of downregulation of hepatic Phase II glucuronidation. Liver disease, including cirrhosis and hepatitis can result in downregulation as can hypothyroidism and malnutrition (Lu 2005). Loss-of-function glucuronidation SNPs are also well-recognized, e.g. Gilbert’s Syndrome. This would presumably result in low levels of multiple glucuronides.

Note that females tend to have lower glucuronidation activity compared to males, and older individuals in general tend to form glucuronides less readily.

Low glucuronidation may also reflect a lack of cofactors for the enzyme. A list of supplements and foods that promote glucuronidation can be found in the following article: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4488002/

A long list of medications including azepams, NSAIDs, codeine, amitryptyline, ethinyl estradiol, and anti-seizure medications may inhibit glucuronidation.

CORTISOL GLUCURONIDE FRACTION IS LOW NORMAL OR LOW
The proportion of hydrolyzed cortisol (total cortisol by GC-MSMS) lies close to or below the 33rd percentile. The following summarizes supplements which might increase cortisol glucuronide (and decrease free cortisol). From Hodges 2015:

Resveratrol
Dandelion
Rooibos
Rosemary
Soy
Ellagic acid
Curcumin

Again, from Hodges, the following factors may lower cortisol glucuronide, thereby causing the low percent cortisol glucuronide issue to arise in the first place:

High fat diet
Green or black tea
Quercetin (red onions)
Rutin
Naringenin
Peppermint oil
Cacao
Silymarin
Lithocholic acid, chenodeoxycholic acid (Lu 2005)
Astaxanthin
Calcium glucarate
Oligomeric Proanthocyanins (OPC)

11-BETA HSD2 ACTIVITY RATIO IS IN THE NORMAL OR ACTIVE ZONE
The marker for the activity of the 11-beta HSD2 enzyme is in the active zone which simply means that the enzyme is actively converting cortisol to cortisone. Free cortisone is typically somewhat greater than free cortisol but not excessively so. This is quite normal. The interpretation is that 11-beta HSD2 is neither markedly inhibited nor markedly upregulated/overactive. The derivation of this marker and the rationale for its use is outlined in the Interpretive Guide.

TOTAL CORTISONE RESULT IS ELEVATED
The cortisone result lies above the 66th percentile. Cortisone is consumed /converted to cortisol in the periphery (skeletal muscle/adipose) and in the liver through the activity of the 11-betaHSD1 enzyme. Elevated cortisone may therefore indicate inhibition of 11-betaHSD1. Keeping cortisone as cortisone prevents clinical manifestations of glucocorticoid excess.

Inhibitors of 11-betaHSD1 include: natural compounds e.g. apigenin and quercetin found in a wide variety of vegetables, fruits and spices (Zhu 2018). as well as estradiol and Growth Hormone (Walker 2001), testosterone (Gambineri 2014) and certain bile acids (Diederich 2000).

There is a dose-dependent effect of the bile acid CDCA on the activity of the 11beta-HSD enzymes (both type 1 and type 2 can be affected) (Morris 2004). The activity of AKR1D1 is also influenced by CDCA as mentioned in the Preamble. Bile acids, in turn, are entangled with thyroid hormone activity.

The clinician has to give careful consideration to the thyroid axis along with the capacity of the liver to form and secrete bile when attempting to understand disturbances in the cortisol-cortisone balance.

STEEP CORTISOL DAY CURVE
The day curve slopes steeply down to the right and its slope is -5.2 ng/mg creatinine-hour. A curve of this nature was seen in fewer than 1 in 5 people in our reference population.

A central aspect of the day curve is the area under the curve or AUC. The sum of the free cortisol concentrations in the three samples is proportional but not identical to the area under the curve-reflecting free cortisol output. Here the result for the AUC lies in the upper tertile possibly reflecting overall higher free cortisol output and/or a point which is higher than usual.

The rise in cortisol overnight is attenuated; the degree of increase is in the bottom tertile of the reference population. More detailed commentary on the attenuated overnight rise in cortisol will follow.

Treatment will depend on the clinical correlation and whether the patient is exhibiting signs and symptoms of excess tissue exposure to cortisol.

BEDTIME CORTISOL AND SLEEP
Recognize that there is an optimum range for bedtime cortisol. If bedtime cortisol is too low, there is some evidence that normal sleep architecture is not established (insufficient REM sleep) (Garcia-Borreguero 2000). Conversely, high bedtime cortisol is suppressive for melatonin, and may result in difficulty initiating and sustaining sleep.

Here the result for free cortisol measured at bedtime is high, lying above the 66th percentile so it may be worthwhile considering whether the free cortisol level before retiring is playing a role in sleep problems for this patient. No complaints about sleep were noted on the requisition but it is still worth questioning the patient about this possibility.

OVERNIGHT CORTISOL RESPONSE IS LOW
The result for the Overnight Cortisol Response or ratio of free cortisol in the overnight (D sample) to free cortisol in the bedtime (C sample) is 1.2 and this lies at or below the 33rd percentile. Note that this result is not displayed graphically on the Cortisol & Cortisol Metabolites Heat Map page but can be appreciated by looking at the relevant bar graphs preceding this block of comments.

A low ratio is typically seen when cortisol secretion is static throughout 24 hours-indicating loss of diurnal variation. Cortisol may be either statically high or statically low and the management depends on which of these two situations applies. A low ratio could also arise if there was a transient, acute emotional or physical stressor in the hours before retiring, which would elevate bedtime cortisol.

Melatonin and cortisol enjoy a reciprocal relationship. Melatonin supplementation may act to suppress the overnight rise in cortisol. Elevated testosterone can also suppress cortisol by enhancing hypothalamic sensitivity to negative feedback inhibition of CRH release.

Note that if the overnight and or bedtime creatinines differ significantly from each other this can distort the appearance of the free cortisol curve. The underlying trends are still valid in most cases.

MORNING CORTISOL RESPONSE IS ELEVATED
The Morning Cortisol Response (MCR) is defined by RMA as the ratio of free cortisol in the Morning or (A) specimen to free cortisol in the Overnight (D) specimen. The ratio is 6.7 for this patient. This result lies above the 66th percentile for the reference population. Note that this result is not displayed graphically on the Cortisol & Cortisol Metabolites Heat Map page but can be appreciated by looking at the relevant bar graphs preceding this block of comments.

The surge in free cortisol measuring in saliva in the first hour after waking is known as the Cortisol Awakening Response (CAR). An elevated saliva CAR has been associated with stress anticipating the upcoming day/week, anxiety and panic disorder, an acute stressor unique to the day of collection, untreated anorexia nervosa, insulin-glucose axis dysregulation, as well as deficiency of omega-3 fatty acids.

The urine MCR discussed here MAY be a surrogate for saliva CAR measurements but additional research is needed. An elevated urine MCR needs to be interpreted in light of a good history surrounding acute and longer term stressors.

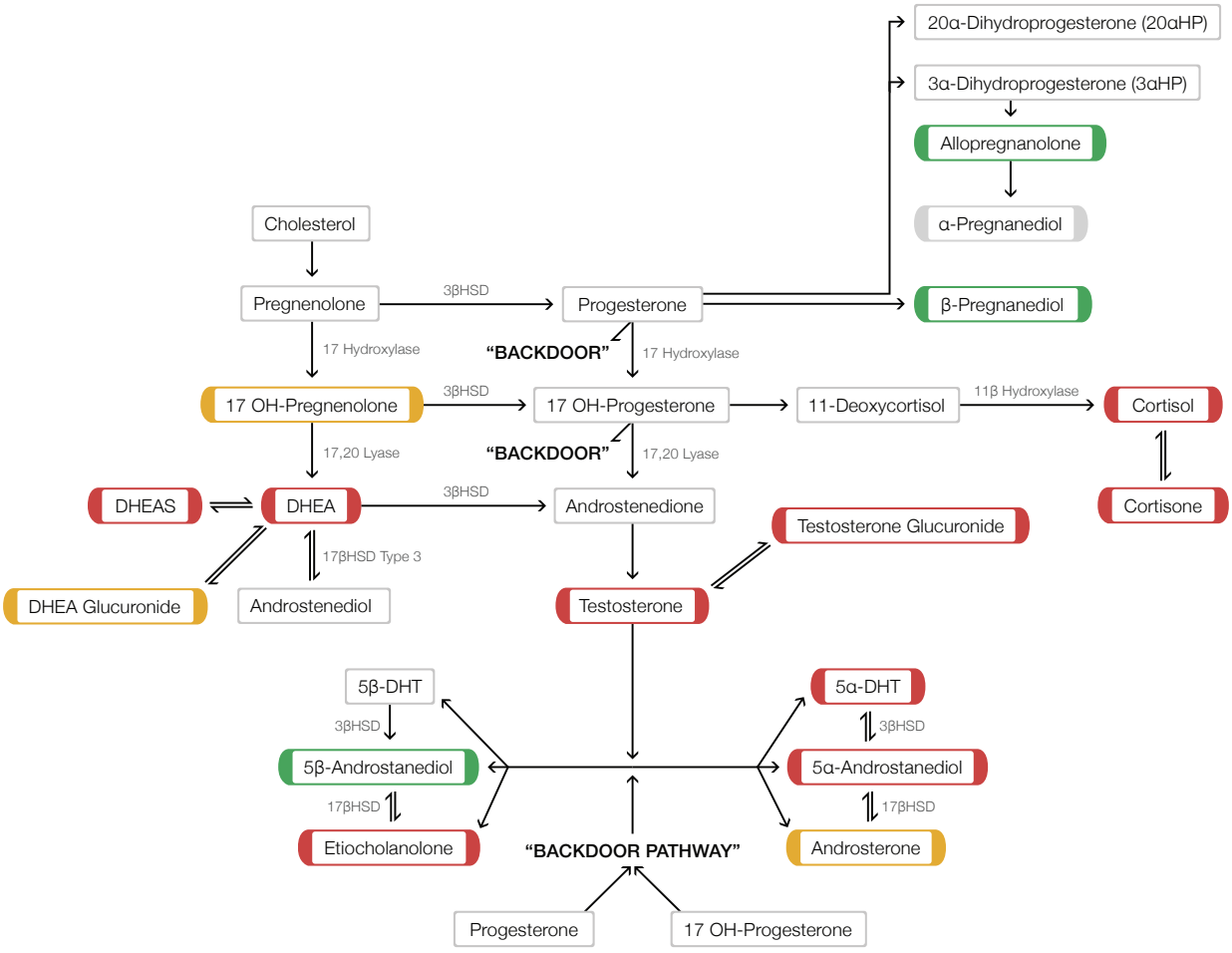
Note that if the overnight and or morning creatinines differ significantly from each other this can distort the appearance of the free cortisol curve. The underlying trends are still valid in most cases.

BALANCED RATIO: CORTISOL/TESTOSTERONE

We consider that the ratio of cortisol to testosterone might be reflective of the balance between anabolic and catabolic steroid hormones. This result is displayed in the bar graph section and also on the first page of the report. When the indicator points within the middle 1/3 of the semicircular gauge (between the 33rd and 66th percentiles) this may reflect a reasonable balance between tissue breakdown (catabolism) and tissue growth/repair (anabolism).

The ratio is constructed from molar concentrations (nmol/mL):
Cortisol * / (Testosterone + DHT + alpha-Androstanediol + beta-Androstanediol)
* total Cortisol via GCMSMS after hydrolysis

PROGESTERONE & 17OH PRECURSOR STEROIDS
Hormone Heat Map
HMUS04



PROGESTERONE METABOLITES

Analyte	Result	Range	Units	0% 16% 33% 66% 84% 100%	%ile	Range Applied
17-Hydroxypregnenolone	2.9	1.2 - 4.2	ng/mL		69%	d21
a-Pregnanediol + ß-Pregnanediol	1,300	370 - 4,000	ng/mL		38%	d21
ß-Pregnanediol	1,300	370 - 4,000	ng/mL		38%	d21
a-Pregnanediol	< 100	0 - 0	ng/mL		<DET	No data
ß-Pregnanediol/a-Pregnanediol	< 0.010	0 - 0	ng/mL		<DET	No data
Allopregnanolone	83	18 - 340	ng/mL		41%	d21
Free Progesterone (via GCMSMS)	0.54	0.30 - 1.5	ng/mL		47%	d21

17-HYDROXYPREGNENOLONE RESULT IS ELEVATED
The result for 17-hydroxypregnenolone (17HPE) lies above the 66th percentile. The median age of the population upon which our luteal phase reference range was based is approximately 40 years. Results for younger women will therefore tend to fall into the higher percentiles, as 17HPE declines with age. This patient's result might be normal for age if she is younger than 30 years.

These are the average 17-hydroxypregnenolones by decade, in our database:

<30 years: 3.3 ng/mL
31-40 years: 2.8 ng/mL
41-50 years: 2.2 ng/mL
>50 years: 1.4 ng/mL

Elevated 17HPE levels have been noted in normo- and hyperandrogenic women with polycystic ovary syndrome (de Medeiros 2017). In that study, the women with high androgens had the highest mean serum levels of 17HPE. They also had the highest mean serum insulin, HOMA-IR, triglycerides, hemoglobin A1c, estradiol and DHEAS. In short, elevated 17HPE should raise suspicion of insulin resistance and/or Metabolic Syndrome in the appropriate clinical context (obesity, increased WHR).

It appears from our database, that younger, slender females with normal cycles may tend to have higher 17HPE as a normal finding. Elevated 17HPE is ofen found in conjunction with elevated DHEA, which is also seen to be higher in slender females with regular menses.

Supplementation with pregnenolone, especially orally or sublingually might elevate 17-hydroxypregnenolone.

SUM OF ALPHA- AND BETA-PREGNANEDIOL: ALPHA P2 BELOW REPORTING LIMIT
The result for alpha-pregnanediol is below the reporting limit of 100 ng/mL. In order to report a result for the sum of alpha- and beta-pregnanediols, a value of 50 ng/mL is arbitrarily assigned for alpha-pregnanediol when it is below the reporting limit.

Here the beta-pregnanediol result is 1,300 and the alpha-pregnanediol result is below the reporting limit but we still know that the result for the sum lies somewhere between 1,300 and 1,400 ng/mL so we split the difference and report the sum as 1,300 ng/mL.

This is a rather arbitrary decision and has the effect of making the sum of alpha- and beta-pregnanediol appear higher than the picture gleaned from beta-pregnendiol in isolation.

ALLOPREGNANOLONE RESULT IS NORMAL
The result for allopregnanolone lies between the 33rd and 66th percentiles. Results in this range may not be associated with any particular symptoms but as outlined in the Interpretive Guide, some women who are susceptible may have PMDD symptoms despite relatively normal allopregnanolone levels.

Interventions to ameliorate adverse effects of allopregnanolone in sensitive individuals with normal or elevated allopregnanolone levels include GABA-A receptor antagonists such as Sepranolone (epiallopregnanolone) as well as 5-alpha reductase inhibitors such as finasteride and saw palmetto.

Metabolic syndrome and insulin resistance are associated with increased activity of 5-alpha reductase. Addressing insulin-glucose abnormalities might be of benefit in some situations.

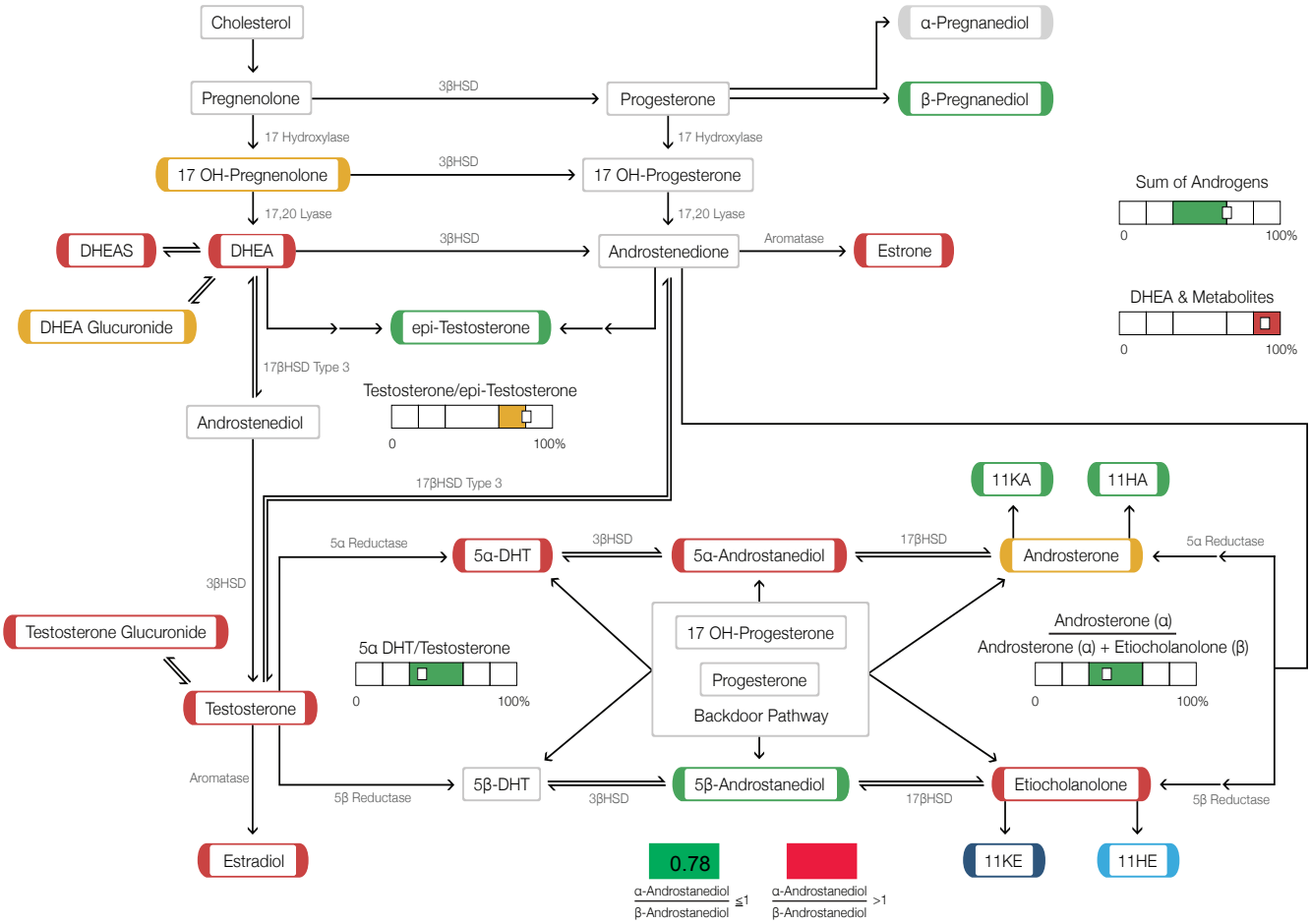
alpha-PREGNANEDIOL BELOW LIMIT OF REPORTING
The result for alpha-pregnanediol is below the limit of reporting (very low). This is the case for 63% of the women in the luteal phase, in our reference population. In other words, 2/3 of normal females in the luteal phase do not make a measurable amount of this analyte.

There is almost nothing in the literature regarding alpha-pregnanediol. The clinical significance of this result is unknown, beyond that it is reflective of overall low progesterone production.

RATIO beta-PREGNANEDIOL/alpha-PREGNANEDIOL CAN'T BE CALCULATED
The ratio of beta- to alpha-pregnanediol isn't calculated because the result for alpha-pregnanediol is below the limit of reporting.

FREE PROGESTERONE RESULT IN PHYSIOLOGIC RANGE
The result for free progesterone measured via GC-MSMS is within the physiologic range.

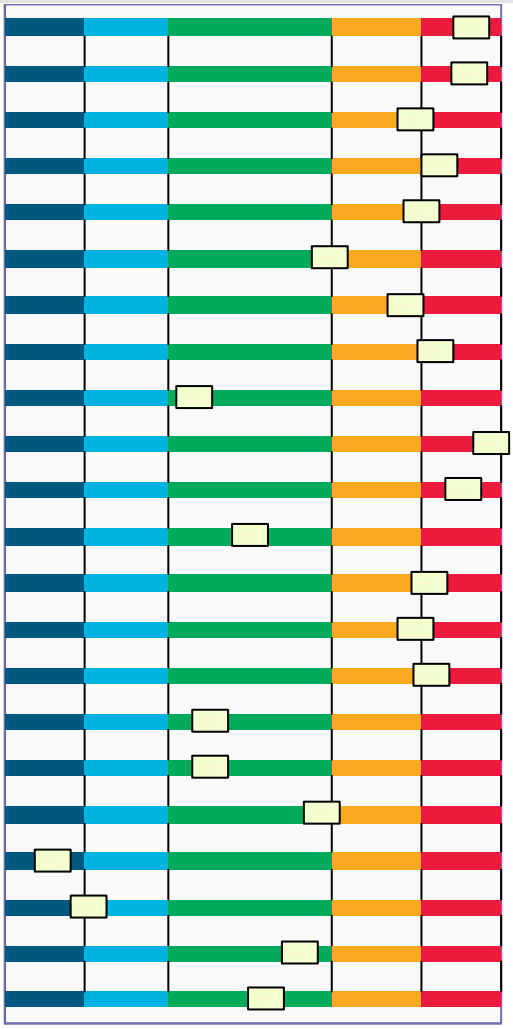
ANDROGENS/17-KETOSTEROIDS
Hormone Heat Map
HMUS05



ANDROGENS / 17 KETOSTEROIDS

ANDROGEN RATIOS AND SUMS

Analyte	Result	Range	Units	0% 16% 33% 66% 84% 100%	%ile	Range Applied
DHEA (free and conjugated)	1,200	160 - 640	ng/mL		94%	d21
DHEA sulphate (DHEAS) via LCMSMS	1,300	66 - 560	ng/mL		94%	d21
DHEA glucuronide via LCMSMS	300	100 - 280	ng/mL		83%	d21
DHEA&metabolites (DHEA+AND+ETIO)	4,700	1,500 - 3,400	ng/mL		88%	d21
Testosterone (T)	10	1.3 - 9.2	ng/mL		84%	d21
Epi-testosterone (Epi-T)	22	12 - 31	ng/mL		66%	d21
Testosterone/Epi-Testosterone (T/Epi-T)	0.46	0.078 - 0.44			81%	d21
a-Dihydrotestosterone (aDHT)	4.1	0.49 - 3.8	ng/mL		87%	d21
a-DHT/Testosterone	0.39	0.26 - 0.85			38%	d21
Testosterone glucuronide via LCMSMS	11	0.60 - 5.8	ng/mL		98%	d21
a-Androstanediol	30	8.7 - 25	ng/mL		93%	d21
ß-Androstanediol	38	15 - 74	ng/mL		50%	d21
a-Androstanediol/ß-Androstanediol	0.78	0.24 - 0.68			86%	d21
Androsterone (AND)	1,500	450 - 1,400	ng/mL		83%	d21
Etiocholanolone (ETIO)	2,100	650 - 1,600	ng/mL		86%	d21
Androsterone Excretion Ratio	0.41	0.35 - 0.53			42%	d21
AND/ETIO	0.70	0.54 - 1.1			42%	d21
Androgen sum (T,DHT,androstanediols)	82	27 - 110	ng/mL		64%	d21
11-Ketoetiocholanolone (11KE)	56	91 - 300	ng/mL		9.9%	d21
11-Hydroxyetiocholanolone (11HE)	71	79 - 450	ng/mL		17%	d21
11-Ketoandrosterone (11KA)	15	8.4 - 21	ng/mL		60%	d21
11-Hydroxyandrosterone (11HA)	360	240 - 580	ng/mL		53%	d21



DHEA RESULT IS ELEVATED
The result for DHEA (measured by GC-MSMS after sample hydrolysis) lies above the 66th percentile.

High or high normal DHEA may be the result of supplementation with DHEA, pregnenolone or growth hormone. Some homeopathics and herbal adaptogenic supplements are also known to elevate DHEA. Supplementation with topical or oral progesterone may result in high normal/elevated DHEA in some women, although the mechanism is uncertain. In men and women, elevated DHEA levels are associated with central adiposity. A variety of neoplasias affecting multiple points along the HPA axis may also lead to elevated DHEA.

The clinical picture of high DHEA can include insulin resistance/metabolic syndrome in women of any age. In younger women, high DHEA may be associated with irregular or absent menses as well as polycystic ovary syndrome (PCOS). The connection between increased DHEA, insulin resistance and increased Body Mass Index (BMI) is not absolute. Many women with high DHEA have insulin resistance and elevated BMI, but high DHEA/S can still be associated with insulin resistance when BMI is normal. Elevated DHEA may also be seen in lean, athletic women who are otherwise healthy.

Elevated DHEA may be associated with symptoms of either high androgens (oily skin, hair loss, irritability, acne) or high estrogens (weight gain at hips, breast tenderness, fluid retention, heavier menses, migraines) depending on which way DHEA is metabolized. Any given individual may not have every one of these symptoms and some individuals with high DHEA do not have any of these symptoms.

Weight reduction, low refined carbohydrate intake and insulin sensitizers such as metformin may be helpful in some cases. Supplementation with EPA (fish oil) may help regulate steroidogenesis if insulin resistance/hyperinsulinemia is present. The best course of action should be left up to the treating practitioner if DHEA is elevated but there are no high androgen symptoms and a normal insulin/glucose axis.

BODY MASS INDEX (BMI) <= 24
Women who are slender or who tend toward the slender end of the spectrum often have high levels of DHEA and DHEA metabolites and in many cases, these women do not have any symptoms of elevated androgens. Body Mass Index or BMI serves as a rough guide in this regard. Some patients with a BMI in the range 22 to 24 may have high androgens, and this is quite common in women whose BMI is less than 22. This patient's BMI is 22.

Elevated DHEA may be part of the reason why some women are slender, since DHEA is associated with fat-burning. DHEA also helps maintain bone density, muscle mass and may help dispose glucose into muscle by a non-insulin dependent mechanism. Hence elevated DHEA and metabolites may confer significant health benefits in the right patient.

TESTOSTERONE RESULT IS ELEVATED
The result for testosterone lies above the 66th percentile. This may be associated with skin symptoms of elevated testosterone such as oily skin and acne. Scalp hair loss may be noted. Some women become more irritable when testosterone is elevated. Note that the presence or absence of symptoms depends on many factors and may not always be correlated solely to one hormone level. The effect of high testosterone may be counterbalanced or opposed by cortisol and estrogens.

Before menopause, about half the testosterone in the body comes from the ovaries. The other half is made from DHEA of adrenal origin. Hence elevated testosterone may be reflective of elevated DHEA. Elevated testosterone may be associated with Metabolic Syndrome, insulin resistance and PCOS (younger women). Some slender, athletic women have elevated testosterone and this may persist well into old age.

A variety of supplemented hormones (apart from testosterone itself) may elevate testosterone: these include progesterone, DHEA and pregnenolone.

DHEA AND DHEA METABOLITE RESULTS ARE ELEVATED
The approximate mass balance for DHEA can be assessed by looking at the sum of the results for DHEA and its metabolites androsterone (5-alpha reduced metabolite) and etiocholanolone (5-beta reduced metabolite). Here the result for that sum lies close to or above the 66th percentile. This reinforces the notion that overall production of DHEA is elevated.

Note that if other hormones such as progesterone, pregnenolone, 7-keto DHEA and testosterone are supplemented this may result in elevated androsterone and etiocholanolone. Elevated 17-hydroxypregnenolone can also convert to DHEA metabolites via 17-hydroxyprogesterone, without elevating DHEA.

DHEA and its metabolites, in aggregate, may be elevated in the setting of Metabolic Syndrome/Insulin resistance. If the BMI is circa 22 or lower, this is less likely, as discussed.

TESTOSTERONE GLUCURONIDE RESULT IS ELEVATED
Intact testosterone glucuronide is measured directly by LC-MSMS. When the sample is hydrolyzed for GC-MSMS analysis all testosterone glucuronide should be converted to free testosterone. The GC result should represent the total of free testosterone naturally present, free testosterone generated from hydrolysis of testosterone glucuronide, and free testosterone generated by hydrolysis of testosterone sulfate. The GC and LC results are not additive. Typically the testosterone result should be about 0.6 to 0.8 times the testosterone glucuronide result, if free testosterone and testosterone sulfate levels are minimal.

Here, an elevated testosterone glucuronide result is a simple reflection of increased total testosterone production.

EPITESTOSTERONE RESULT IS AVERAGE
The result for epitestosterone lies between the 33rd and 66th percentiles; this is an average result. Epitestosterone arises primarily from the testes in males with normal testicular output. Epitestosterone in females has not been well-studied.

RATIO: TESTOSTERONE/EPITESTOSTERONE IS ELEVATED
The result for the ratio testosterone/epitestosterone lies above the 66th percentile. This ratio is sensitive to testosterone supplementation because epitestosterone does not change when testosterone is supplemented. As a result, even small increases in testosterone will be amplified by taking the ratio to epitestosterone

Normally when testosterone doping occurs in athletes via IM testosterone, the ratio of testosterone to epitestosterone rises to at least 2 or 3 although this is dose-dependent.

The implication of a higher than normal ratio is that even if testosterone remains at a low range, it still may have been influenced by supplementation.

ALPHA DHT RESULT IS ELEVATED
The result for 5-alpha DHT lies above the 66th percentile. The activity of the 5-alpha reductase enzyme may be upregulated. Increased tissue activity of T3 is known to upregulate this enzyme. If this individual is supplementing with thyroid hormones/thyroid support it might be worth revisiting the clinical presentation. Zinc deficiency can lead to increased 5-alpha reductase activity.

In women, upregulated 5-alpha reductase activity may also be seen in insulin resistance and PCOS (Wu 2017).

DHT may arise through the so-called Backdoor Pathway as well (Kamrath 2012). If this is likely, there will be additional commentary elsewhere which explains the pathway.

Signs of elevated 5-alpha DHT such as acne, oily skin and increased facial hair growth might be present.

Measures to reduce the conversion of testosterone to alpha DHT include mitigation of insulin resistance depending on the presentation. The enzyme activity may be reduced or blocked by medications intended to reduce the symptoms of an enlarged prostate, as well as by isotretinoin (Accutane). Especially in females, note that pharmaceutical inhibition of hepatic 5-alpha reductase can lead to fatty liver (Hazlehurst 2016) since reduction is a key mechanism whereby cortisol is irreversibly inactivated in the liver.

Other 5-alpha reductase substrates include progesterone and androstendione. Supplementation with progesterone in very modest doses may compete with testosterone and reduce DHT formation. (This is more commonly used in males with prostate issues.) Epitestosterone is also known to be an inhibitor of 5-alpha reductase (Choi 2001).

Supplements including zinc, beta-sitosterol, and various medium and long chain fatty acids (GLA, coconut oil, oleic acid) may inhibit/reduce the activity of 5-alpha reductase. There are a variety of herbs/plant extracts which also do so, including saw palmetto, green tea and Reishi mushroom (Grant 2012).

RATIO: alpha-ANDROSTANEDIOL/beta-ANDROSTANEDIOL <= 1
The androstanediols can be formed either from 5-alpha and 5-beta DHT or from androsterone and etiocholanolone. We measure the ratio of 3-alpha-5-alpha androstanediol to 3-alpha-5-beta androstanediol. In our database, these analytes correlate most closely to androsterone and etiocholanolone so their ratio usually reflects the alpha-beta reductase balance of their "parents". Usually etiocholanolone (beta) is greater than androsterone (alpha), and this carries into the androstanediols where the alpha/beta ratio is almost exclusively less than 1 in females.

This patient's result is normal.

ANDROSTERONE RESULT IS ELEVATED
The result for androsterone lies above the 66th percentile. Various lines of inquiry indicate that the degree of formation of androsterone is sensitive to T3 (triiodothyronine) through upregulation of alpha-reductase. Administration of T3 to male subjects > 70 years of age resulted in increased production of androsterone (Trout 1970). Skovsted also noted a positive correlation between androsterone and thyroid status (Skovsted 1966). Ueshiba found that elevated androgens in hyperthyroid women declined progressively with treatment (Ueshiba 1997).

Further investigation might be warranted if there are signs and symptoms of elevated tissue activity ot T3. Bear in mind that upregulated activity of alpha-reductase might be seen in normal,athletic women with elevated levels of 17-hydroxypregnenolone and DHEA.

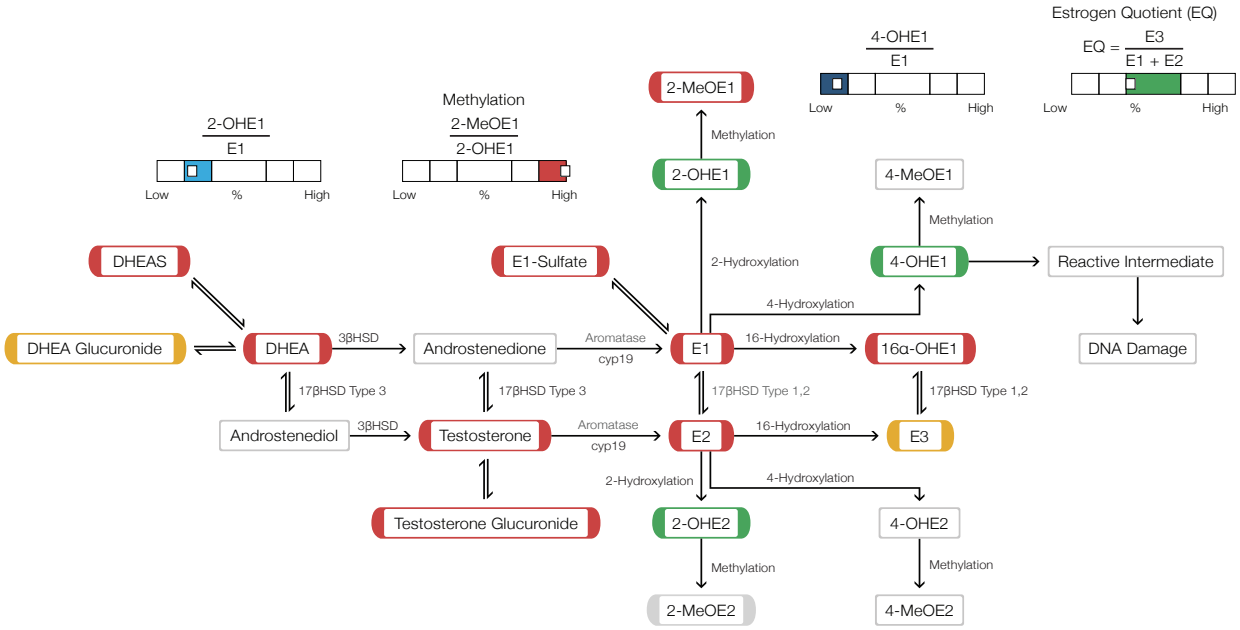
Note that androsterone and androstanediol are interconvertible through the 17-beta hydroxysteroid dehydrogenase enzyme system. Elevated androsterone might be accompanied by elevated androstanediol. Androstanediol is associated with hirsutism (Falsetti 1998).

Note also that if the patient is supplementing with DHEA we can expect that androsterone and/or etiocholanolone might be elevated. This can feed through to alpha-DHT without involving testosterone.

RESULT FOR ANDROSTERONE EXCRETION RATIO IS NORMAL
The ratio Androsterone/(Androsterone + Etiocholanolone) can be referred to as the Androsterone excretion ratio. This ratio is sensitive to the activity of 5-alpha reductase as Androsterone is a 5-alpha reduced metabolite of Androstenedione. 5-alpha reductase is in turn, sensitive to thyroid hormone activity, being downregulated when thyroid activity is low (Skovsted 1966).

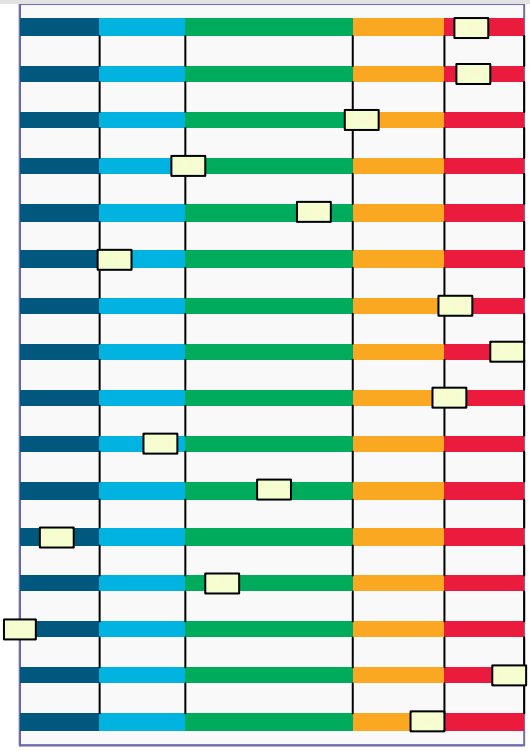
Here the result lies comfortably within the middle tertile and is more or less average.

ESTROGENS & METABOLITES
Hormone HeatMap
HMUS07



ESTROGENS

Analyte	Result	Range	Units	%ile	Range Applied
Estrone (E1)	20	5.3 - 15	ng/mL	89%	d21
Estradiol (E2)	7.8	2.3 - 5.5	ng/mL	90%	d21
Estriol (E3)	27	6.7 - 39	ng/mL	68%	d21
E3/(E1 + E2)	0.95	0.65 - 2.7		33%	d21
2-Hydroxyestrone (2-OHE1)	7.5	3.0 - 12	ng/mL	58%	d21
2-OHE1/E1	0.37	0.38 - 0.99	ng/mL	19%	d21
2-Methoxyestrone (2-MeOE1)	5.6	0.79 - 4.5	ng/mL	86%	d21
2-MeOE1/2-OHE1	0.74	0.21 - 0.55		97%	d21
16a-Hydroxyestrone (16a-OHE1)	12	2.3 - 10	ng/mL	85%	d21
2-OHE1/16a-OHE1	0.65	0.43 - 2.6		28%	d21
4-Hydroxyestrone (4-OHE1)	1.2	0.53 - 2.1	ng/mL	50%	d21
4-OHE1/E1	0.057	0.082 - 0.20		7.3%	d21
2-Hydroxyestradiol (2-OHE2)	0.77	0.42 - 2.8	ng/mL	40%	d21
2-Methoxyestradiol (2-MeOE2)		0 - 0	ng/mL	<DET	No data
Estrone sulphate (E1-Sulphate or E1S)	8.9	0.45 - 2.0	ng/mL	97%	d21
Sum of Estrogens	81	27 - 80	ng/mL	81%	d21



8-HYDROXY-2-DEOXYGUANOSINE (8OH2dG) - OVERNIGHT COLLECTION

NOTE: The upper end of the listed range is the 67th percentile which is at the green-yellow border.

Analyte	Result	Range	Units	%ile	Range Applied
8-Hydroxy-2-deoxyguanosine (8-OH2dG)	3.6	0 - 0.43	ng/mg	91%	d21

6-SULFATOXY MELATONIN (OVERNIGHT COLLECTION)

Analyte	Result	Range	Units	%ile	Range Applied
6-Sulfatoxymelatonin	18	6.8 - 22	ng/mg	71%	d21

CREATININE

Analyte	Result	Range	Units	%ile	Range Applied
Pooled Creatinine	0.81	0.88 - 1.8	mg/mL	12%	d21
Overnight Creatinine (D)	0.85	0.86 - 1.9	mg/mL	18%	d21
Morning Creatinine (A)	0.72	0.68 - 2.0	mg/mL	23%	d21
Dinnertime Creatinine (B)	0.94	0.37 - 1.4	mg/mL	63%	d21
Bedtime Creatinine (C)	0.83	0.46 - 1.6	mg/mL	41%	d21

Free steroids : USEFUL FOR DETECTION OF CONTAMINATION OF URINE BY SUPPLEMENTAL HORMONES

Analyte	Result	Range	Units	%ile	Range Applied
Free Estrone (via LCMSMS)	< 0.50	0.41 - 0.93	ng/mL	<DET	d21
Free Estradiol (via LCMSMS)	< 0.60	0 - 0.65	ng/mL	<DET	d21
Free Estriol (via LCMSMS)	< 0.50	0 - 0.60	ng/mL	<DET	d21
Free Testosterone (via LCMSMS)	< 0.90	0.76 - 0.93	ng/mL	<DET	d21
Free Progesterone (via GCMSMS)	0.54	0.30 - 1.5	ng/mL	47%	d21

FINDINGS ARE CONSISTENT WITH INCREASED AROMATASE ENZYME ACTIVITY

Cortisol induces increased synthesis of the aromatase enzyme which converts androgens to estrogens. Note also that since cortisone may be converted back to cortisol in adipose tissue, both cortisol and cortisone may be drivers of increased aromatase activity.

The results for cortisol, cortisone, testosterone, estradiol and estrone are all elevated above the 66th percentile. This pattern is consistent with increased aromatase activity.

Measures to mitigate increased conversion of androgens to estrogens include zinc supplementation, lowering of cortisol and cortisone levels, melatonin supplementation and weight loss.

TOTAL ESTROGENS RESULT IS HIGH NORMAL OR HIGH

The result for the sum of all the estrogens is close to or above the 66th percentile. Remember that the estrogens measured here came into urine as conjugates. High levels of conjugates do not necessarily translate into high estrogen symptoms. (High normal or high estrogens may be associated with complaints including breast tenderness, fibrocystic breasts, heavy menses, migraines and uterine fibroids.)

NOTE: Elevated estrogens burden the liver causing thickened bile, gallbladder problems and potential elevation in bile acids. This can interfere with clearance of cortiol and cortisone to the tetrahydro- metabolites which in turn, may negatively impact the HPA axis and production of cortisol.

High or high normal estrogens can be seen with recent use of estradiol and/or estrone replacement, DHEA supplementation, pregnenolone supplementation, particularly oral, sublingual or troche supplementation of any of these hormones. Patients sometimes neglect to list hormones they might be supplementing.

Some herbal supplements such as Vitex and Wild Yam elevate LH, which can increase the estradiol level. Clomid may also increase estradiol by elevating LH. HCG mimics the effect of LH on the ovaries.

Other factors affecting estrogen levels include elevated percent body fat, constipation (increased recycling of estrogens), elevated cortisol (upregulated aromatase) and excessive consumption of commercially raised beef and poultry (high in estrone/estrone sulphate).

Testosterone supplementation may result in elevated estrogens through conversion of testosterone to estradiol and estrone via the aromatase enzyme system. High endogenous testosterone may result in high estrogens for the same reason (increased aromatization).

Use of exogenous corticosteroids may elevate estrogens through induction of aromatase. High estrogens may also be a manifestation of sluggish clearance of estrogens by the liver. Note that in overweight women, the attendant increased risk of breast cancer has been found to be related to the elevated estradiol levels. (Key 2003).

Other factors involved with increased risk of breast cancer include hyperinsulinemia/insulin resistance (usually accompanied by elevated androgens), high bedtime cortisol, low nocturnal melatonin production, and breakdown of estrogen into unwanted estrogen metabolites.
Symptoms of estrogen excess may often be relieved by supplementation with progesterone, even in the face of normal levels of progesterone or progesterone metabolites.

SUM OF ESTROGENS ELEVATED: PROGESTERONE NORMAL OR LOW
The result for the sum of estrogens is elevated above the 75th percentile whereas the result for the sum of the pregnanediol analytes is below the 60th percentile. This admits the possibiolity that the patient might have complaints of estrogen-progesterone imbalance (breast tenderness, fibrocystic breasts, headaches, fluid retention). The patient left the symptom inventory portion of the requisition blank.

SUM OF ESTROGENS AND TOTAL DHEA IS ELEVATED
Here, the result for the total of all estrogens measured along with total DHEA ranks at or above the 80th percentile. In general, estrogens can arise peripherally from testosterone and androstenedione, respectively, via the aromatase enzyme expressed in adipose tissue as well as the liver. High DHEA can therefore lead to elevated estrogens, as may be the case here.

Factors which may increase aromatase activity include obesity, especially visceral obesity. If an inflammatory milieu exists within adipose tissue, the cytokine mixture produced tends to increase aromatase activity.

Zinc deficiency can also play a role. Zinc (and progesterone) are often cited as "aromatase inhibitiors", but both zinc and progesterone do not inhibit the activity of the aromatase enzyme. They do decrease the transcription of the gene coding for aromatase, resulting in a net decrease in the number of copies of the enzyme produced in any given tissue. Insufficent zinc (and progesterone) takes the "brakes" off the espression of aromatase enzyme copies. Similarly, melatonin acts as a brake on aromatase expression. Low melatonin was promote higher estrogens.

The patient did not list the use of estrogens, testosterone, DHEA, 7-keto DHEA or pregnenolone. It's quite possible that her estrogens are high because she is using one or more of these hormones, but didn't list this on the requisition.

DHEA, ESTRADIOL AND ESTRONE ELEVATED
The results for estradiol and estrone lie above the 66th percentile and endogenous DHEA is elevated above the 84th percentile. DHEA may be converting to estrogens via excessive aromatase activity.
Whether measures to throttle back aromatase should be taken will depend on clinical correlation: presence of symptoms and signs of estrogen excess.

ESTRONE SULFATE RELATIVE TO ESTRONE
For this patient, estrone sulfate comprises more than 25% of total estrone on a weight-weight basis. This proportion is seen in fewer than 20% of the women in our reference populations, as normally most estrone is present in urine as the

glucuronide conjugate. (Note that the estrone sulfate and estrone results are not additive. The estrone result reflects innate free estrone as well as free estrone arising from hydrolysis of estrone sulfate and glucuronide. The estrone sulfate result is "contained" within the estrone result.)

Sulfation of estrogen may be upregulated by other factors including supplementation with thyroid hormones, melatonin, Vitamin D3 and uncontrolled inflammation. Oral estradiol or DHEA supplementation can result in large increases in the proportion of urine estrone sulfate due to rapid biotransformation and conjugation within the proximal small intestine.

Estrone sulfate is regarded as a storage form of estrogen but there are numerous cell membrane transporters capable of importing conjugated molecules, including sulfates, into cells. An elevated level of estrone sulfate could conceivably be associated with a clinical picture of elevated estrogens due to intracellular regeneration of estrone in target cells.

RESULT FOR 16-HYDROXYESTRONE HIGH NORMAL OR HIGH
The result for 16-hydroxyestrone lies close to or above the 66th percentile. Formation of 16-hydroxyestrone is catalyzed primarily by the cytochrome enzyme CYP1A2. Inducers of this enzyme include soluble fibre, flaxseed (Sturgeon 2010) and caffeine. Individuals of African-American descent also have higher levels of this metabolite (Jernstrom 2003).

Elevated 16-hydroxyestrone has been associated with an increased risk of high risk Wolfe-type mammographic parenchymal patterns (P2/DY) in postmenopausal women (Riza 2001).

Elevated 16-hydroxyestrone has also been noted in SLE and Rheumatoid Arthritis (Cutolo 2004). Presumably other high IL-6, high NF-kappaB states might have the same effect, although this has not been well-studied.

Markedly elevated levels of 16-hydroxyestrone might also be due to estrogen or DHEA supplementation (especially oral supplementation) which may or may not have been recorded on the requisition accompanying this sample.

Reduced T3 activity can also shunt estrone toward the 16-hydroxyestrone and away from the 2- and 4-hydroxyestrones (Doufas 2000).

ESTRIOL (E3) ELEVATED
The result for estriol lies above the 66th percentile. In general, elevated estriol is not thought to be harmful since estriol can mitigate the effects of the more potent estradiol, at the receptor level. Most often, elevated E3 will also be reflected by a high value for the Estrogen Quotient: E3/(E1 + E2). E3 and 16-hydroxyestrone are significantly correlated in our reference range population; 16-hydroxyestrone and E3 both tend be be elevated or both tend to be low as they are formed by the same enzyme which 16-hydroxylates E1 and E2.

The patient didn’t list any hormone therapies containing estriol but she may have neglected to list her hormone therapies on the requisition. Licorice contains small amounts of estriol. Some over the counter wrinkle creams and high-end anti-aging face creams have been found to be illegally adulterated with estriol. It is often worth reviewing all skincare products used by a patient with high E3.

The EQ isn't automatically high when E3 is high; it depends on the relative amounts of E1 and E2 that are present.

Iodine supplementation is one way to elevate estriol (Wright 2010). This may be a dose-dependent effect; excessive iodine may result in suppression of thyroid hormone synthesis and a lowering of T3. Reduced T3 in turns shunts estrogens toward E3 and 16-hydroxyestrone at the expense of 2- and 4-hydroxyestrogens (Doufas 2000).

One would not give iodine to attempt to raise the EQ, when estriol is already high. Iodine is more appropriately given when E3 is low.

EFFECT OF METHYLATION ON INTERPRETATION OF 2OHE1/E1 RATIO
Here the estrone methylation ratio (2-methoxyestrone/2-hydroxyestrone) is higher than average. This makes the apparent ratio 2OHE1/E1 appear lower than it might otherwise be. A low ratio 2OHE1/E1 can only reflect "pure" 2-hydroxylation activity when methylation activity is within normal limits.

RATIO: 2OHE1/E1 RESULT IS LOW NORMAL OR LOW
The result for the ratio 2-hydroxyestrone/estrone (2OHE1/E1) lies close to or below the 33rd percentile. The 2-hydroxylation pathway is considered to be the most favorable hydroxylation pathway, so a normal or even an elevated ratio is not

considered to be detrimental. Concern arises when the relative amount of 2-hydroxyestrone is low relative to estrone as this may indicate an impairment in the ability to eliminate estrogens.

We have known since the 1960's that the conversion of estrone (E1) to 2-hydroxyestrone (2OHE1) is markedly affected by thyroid hormone activity. Low thyroid hormone activity is reflected by low 2OHE1 relative to E1. Conversely, increasing thyroid hormone activity will elevate 2OHE1 relative to E1.

Progesterone is also an important stimulus to 2-hydroxylation. This patient's beta-pregnanediol is low: close to or below the 33rd percentile. In this case supplementation with progesterone might help increase formation of 2-hydroxyestrones.

There are various other measures that can increase flow down the 2OHE1 pathway including, Oil of Rosemary, exercise, DIM, I3C and cruciferous vegetables.

COMMENTARY: 2-16 RATIO (2OHE1/16OHE1)
This ratio is not graphed on the estrogen heat map page; it is found in the bar graphs which follow the heat map. The 2-16 ratio for this patient is 0.65.

The original research on this ratio was performed with an ELISA kit which was not able to distinguish between various 2-hydroxylated estrogens. Therefore, the threshold ratio of approximately 2 pertaining to decreased risk of breast cancer does not apply when considering data generated by mass spectrometric techniques. Prospective studies would have to be undertaken, to determine what relationship, if any, exists between breast cancer and 2- and 16-oxidized estrones measured by GC-MSMS or LC-MSMS.

Practitioners have also assumed that the same logic regarding the 2-16 ratio applies whether the patient is supplementing with estrogens, or making her own, the argument being "estrogen is estrogen". It would be naive and possibly dangerous in the long term to assume that a 2-16 ratio of 3 means the same thing when it is derived from 300/100 (supplemented case) compared to 12/4 (unsupplemented case). The absolute levels of the metabolites in question matter, independently of their ratio. Markedly elevated levels of estrogens are potentially harmful, regardless of any ratio we might concoct between them

Finally, a 2011 meta-analysis by Obi looked at all relevant published dataand concluded that although the ELISA 2-16 ratio might be very weakly related to breast cancer in premenopausal women, it is unrelated to risk of breast cancer in postmenopausal women (Obi 2011).

In summary, the validity of this ratio is somewhat suspect, especially when derived via GCMSMS analysis; nevertheless, for information purposes only, we have elected to display the ratio for the convenience of those who wish to use it.

ESTROGEN QUOTIENT
The Estrogen Quotient is defined as EQ = E3/(E1 + E2). This patient's EQ is 0.95.
In non-Caucasian populations with a low incidence of breast cancer, relative estriol excretion in urine tends to be higher, with an EQ above unity. Conversely, in North America, Caucasian women have an increased risk of breast cancer, and they tend to excrete relatively less estriol in urine, with an EQ closer to 0.5 (Lemon 1966, Lemon 1997).

Some authorities, notably Jonathan Wright MD, maintain that efforts should be made to increase the EQ above unity, to decrease the risk of breast cancer. Wright notes that supplementation with Lugol's iodine will generally accomplish this (Wright 2010).

This effect may be mediated through a suppressive effect of supraphysiologic iodine dosing on thyroid hormone levels. Hypothyroidism is known to increase estriol and 16-hydroxyestrone at the expense of catecholestrogens (Doufas 2000).

Conversely, increased thyroid hormone activity tends to push estrogen toward 2-hydroxyestrogen and away from 16-hydroxyestrone and estriol.

4-HYDROXYESTRONE RESULT IS AVERAGE
The 4-hydroxyestrone result lies between the 33rd and 66th percentile, in the middle third of the reference population. Although this metabolite may be associated with an increased risk of breast cancer, an average level may not be of concern, depending on the context.

If estrogens are being supplemented an average level of 4-hydroxyestrone may be of less concern if the ratio of

4-hydroxyestrone to 2-hydroxyestrone is low despite elevated 2-hydroxyestrone. This suggests that conversion to 4-hydroxyestrone is less vigorous even in the face of elevated estrogens due to supplementation. Ideally the 4-hydroxyestrone result is as low as possible in every circumstance.

RESULT FOR 4OHE1/E1 IS LOW NORMAL OR LOW
4-hydroxyestrone (4OHE1) is a natural metabolite of estrone (E1): in other words, it is not abnormal to form 4OHE1 and the amount of 4OHE1 should rise apace with the overall production of estrogens. The question is: how much is too much?

One way to address this is to look at the ratio 4OHE1/E1 which reflects the rate of formation of 4OHE1. The principal enzyme involved in this conversion is CYP1B1 which is upregulated by inflammation in general and in particular, by exposure to persistent organic pollutants such as dioxins (Smerdova 2014).

The result for this ratio is low normal or low, meaning that the patient makes a lower than average amount of 4OHE1 for a given amount of E1. This finding is not concerning.

PERCENTAGE BREAKDOWN OF HYDROXYESTRONES
Estrone is metabolized by hepatic Phase 1 detoxification enzymes into the hydroxyestrones. Of these, 2-hydroxyestrone is thought to be a favorable estrogen metabolite: 16-hydroxyestrone is probably neutral: 4-hydroxyestrone is potentially harmful as it may cause DNA damage relevant for estrogen-sensiive tissues.

The following percentages are calculated by adding up the results for the three hydroxyestrogens and calculating the percentage of that total for each. The percentages are gender and menstrual status-specific. The range in each case is displayed as: (20th percentile limit - 80th percentile limit).

2-hydroxyestrone: 37 %. (29% - 66%)
16-hydroxyestrone 57 % (23% - 67%)
4-hydroxyestrone 5.8 %. (6.7% - 12.6%)

2-METHOXYESTRADIOL NOT REPORTED
The estrogen metabolite 2-methoxyestradiol is not currently being reported due to a technical issue with the analyte. None of the other estrogens and estrogen metabolites are affected.

8-HYDROXY-2-DEOXYGUANOSINE RESULT IS ELEVATED
Oxidative stress is a term that describes cellular damage from reactive oxygen free radicals (ROS) and reactive nitrogen species (RNS). When ROS react with DNA, the DNA repair process causes 8-hydroxy-2-deoxyguanosine (8OH2dG) to be released and excreted in urine. Measurement of urinary 8OH2dG is therefore a considered a biomarker of DNA damage and oxidative stress.

Free radical formation is a normal part of aerobic metabolism, and some ROS are needed for proper cellular function. Free radicals form naturally with inflammation and cellular metabolism, but can be increased by environmental factors such as excessive UV light, air pollution, ionizing radiation and smoking. Anti-oxidant molecules found in the body and in food can bind to and neutralize free radicals. However, when free radicals outnumber anti-oxidants, damage to lipid membranes, proteins, and DNA can follow.

Oxidative stress has been implicated in a number of disease states: cardiovascular disease (references: Bonomini 2008, Sinha 2015, Touyz 2011), cancers (Halliwell 2007), depression and Parkinson's disease (Blesa 2015), chronic fatigue (Kennedy 2005). Excessive oxidative stress may also result in a general impairment of mitochondrial steroidogenesis..

The result for 8-hydroxy-2-deoxyguanosine lies above the 66th percentile.

Consideration needs to be given to reducing exposure to offending influences, implementing detoxification strategies and supplementation with antioxidants and precursors supportive of glutathione synthesis (NAC, sulfur-containing foods).

MELATONIN METABOLITE RESULT IS ELEVATED
The result for the principal metabolite of melatonin (6-sulfatoxymelatonin) lies above the 66th percentile. Melatonin is instrumental in maintaining proper diurnal rhythm/sleep wake cycling. There is evidence that melatonin has profound influence on immune function in general and specifically may be protective against estrogen-sensitive cancers. Melatonin is also a neuroprotective brain antioxidant.

An elevated level of the principal melatonin metabolite may reflect a neuroprotective response against elevated cortisol but this remains to be verified.

Note a younger person's endogenous melatonin level might be reported as high normal or high simply because the median age of the reference populations is closer to 40 than to 30 years of age. Melatonin decreases with age.

POOLED CREATININE RESULT IS LOW
The result for pooled creatinine lies at or below the 33rd percentile and the result for the sum of the principal metabolites of cortisol (aTHF, bTHF and bTHE) is elevated.

Since creatinine is a byproduct of muscle breakdown/turnover, low urine creatinine can reflect low muscle mass. Low muscle mass, in turn, can be due to low androgens, hypothyroidism, low protein intake and deconditioning. Low urine creatinine may also be indicative of excessive water intake. Finally, kidney disease may also result in low urine creatinine.

The hormones and metabolites in the cortisol family are reported using creatinine normalization in which the raw result in ng/mL is divided by the creatinine concentration in ng/mg to yield results with units of ng/mg creatinine. This helps to compensate for dilute and concentrated urine. Markedly low pooled creatinine may therefore have a modest amplifying effect, yielding higher percentile scores for this family of steroids relative to the other steroid families.

Please contact the Medical Director at 866 370 5227 to discuss. Additional clinical correlation may be needed.

GENERAL COMMENT: FREE SEX STEROIDS
Most of the hormones circulate in blood as conjugates which are more soluble than non-conjugated steroids. Under normal circumstances, there are only minute amounts of free or non-conjugated steroids in blood, with free cortisol being an exception. The same pattern is seen in urine.

In most cases these urine levels can generally be disregarded for men and also for women not using hormones.

If women are applying sex hormones in the vicinity of the urethral meatus (the opening from which urine comes) or on the labia minora, it's possible that these hormones will directly contaminate the urine leading to falsely elevated results. To a lesser extent, this might also happen with vaginal application of hormones. This situation is analogous to using sublingual hormones and then attempting to measure those hormone levels via a saliva sample: direct contamination of the sample may occur.

FREE PROGESTERONE RESULT IN PHYSIOLOGIC RANGE
The result for free progesterone measured via GC-MSMS is within the physiologic range.

We'd like to know more about the patient experience with our tests and services. Please enter the link below into your browser to complete our short online survey. You could win a $50 gift card!

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George Gillson MD, PhD
Medical Director

Note: The College of Physicians and Surgeons of Alberta considers urine steroid hormone testing and some forms of bio-identical hormone replacement to be complementary medicine. The interpretation comments have not been evaluated or approved by any regulatory body. Commentary is provided to clinicians for educational purposes and should not be interpreted as diagnostic or treatment recommendations. *General treatment suggestions can be found in the Rocky Mountain Analytical Resource Binder.

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