

# Hormone Metabolites in Dried Urine

## Steroid Hormone Metabolism

Steroid hormones are low molecular weight, lipid-like molecules that are synthesized from cholesterol mostly in specialized glands such as the adrenals, ovaries, and testes. Once synthesized, these steroids are released into the bloodstream where they enter target tissues/cells and bind to specific hormone receptors. There, the hormone-receptor complex activates specific gene sites and stimulates production of unique cellular products that regulate cell maintenance, growth, and proliferation. Receptors degrade and the steroids uncouple and either bind another receptor to repeat the process, or reenter the circulation where they pass through the liver and begin the process of elimination from the body. The liver and kidneys are the primary organs that metabolize hormones to prepare them for elimination. Metabolism consists of two parts; phase I and phase II. The enzymes involved in phase I are known as P450s or cytochrome P450s (CYP). Currently, there are 57 known genes coding for the various CYPs. Each individual's enzyme activity may be affected by single nucleotide polymorphisms (SNPs) which may alter the activity of a given enzyme. Because of these differences, every individual is unique, inheriting from each parent the ability, or inability, to metabolize hormones in a form that allows for their elimination. These differences may also be exacerbated by nutritional requirements of individual enzymes and metabolic steps within phase I or II<sup>1</sup>.

Phase I metabolism involves CYPs which use oxygen and NADH to add hydroxyl groups that increase the water solubility of the molecule. In Phase II metabolism, a conjugate such as a methyl group, sulfate or glucuronide is added to the modified steroid, making it more inert, water soluble, and likely to be eliminated by the kidney into urine.

Urine steroid hormone metabolites therefore exist primarily as glucuronide and sulfate phase II conjugates, some of which are methylated. Before these

## Available Tests

### Adrenal Profile:

A picture of adrenal hormone metabolism.

### Estrogen Essential:

A baseline view of estrogen metabolites.

### Estrogen Elite:

Estrogen, progesterone, and select androgen metabolites with BPA.

### Basic Metabolites Profile:

A baseline view of sex steroid metabolite levels, corresponding to Saliva Profile I or Blood Profile I.

### Advanced Metabolites Profile:

Our broadest view of steroid hormone metabolite levels plus diurnal melatonin and BPA.

*See page 2 for a table showing the metabolites included in each profile.*

# Hormone Metabolite Testing

A range of profile options

Urine Metabolites Profile Options*		ADRENAL	ESTROGEN ESSENTIAL	ESTROGEN ELITE	BASIC	ADVANCED
Estrogens	Estradiol (E2)		●	●	●	●
	Estrone (E1)		●	●	●	●
	Estriol (E3)		●	●	●	●
	2-Hydroxy Estradiol (2-OH E2)		●	●		●
	2-Hydroxy Estrone (2-OH E1)		●	●		●
	4-Hydroxy Estradiol (4-OH E2)		●	●		●
	4-Hydroxy Estrone (4-OH E1)		●	●		●
	16 $\alpha$ -Hydroxy Estrone (16 $\alpha$ OH E1)		●	●		●
	2-Methoxy Estradiol (2-MeO E2)		●	●		●
	2-Methoxy Estrone (2-MeO E1)		●	●		●
	4-Methoxy Estradiol (4-MeO E2)		●	●		●
	4-Methoxy Estrone (4-MeO E1)		●	●		●
	Bisphenol A (BPA)			●		●
	Progestogens	Pregnanediol (Pgdiol)			●	●
Allopregnanolone (AlloP)				●	●	●
Allopregnanediol (APgdiol)						●
3 $\alpha$ -Dihydroprogesterone (3 $\alpha$ HP)						●
20 $\alpha$ -Dihydroprogesterone (20 $\alpha$ HP)						●
Deoxycorticosterone (DOC)						●
Corticosterone (Ccn)						●
Androgens	DHEA (D)	●		●	●	●
	Androstenedione (Adione)			●	●	●
	Androsterone (Andro)					●
	Etiocholanolone (Etio)					●
	Testosterone (T)			●	●	●
	Epi-Testosterone (Epi-T)			●	●	●
	5 $\alpha$ -Dihydrotestosterone (5 $\alpha$ -DHT)			●	●	●
	5 $\alpha$ ,3 $\alpha$ -Androstanediol (5 $\alpha$ 3 $\alpha$ )					●
Glucocorticoids	Total Cortisol (TC)	●			●	●
	Free Cortisol x4 (FC x4)	●				●
	Total Cortisone (TCn)	●				●
	Free Cortisone x4 (FCn x4)	●				●
	Tetrahydrocortisol (ThC)	●				●
	Tetrahydrocortisone (ThCn)	●				●
Melatonin x4 (MT6s x4)					●	

\*abbreviations as they appear on test requisitions and test reports

conjugates can be analyzed by GC-MS/MS the glucuronide and sulfate groups must first be enzymatically converted back to their original steroid forms (e.g., estradiol sulfate is converted back to estradiol and testosterone glucuronide back to testosterone). For GC-MS/MS analysis the steroids are present at very low concentration and must be first chemically modified (derivatized) to increase their sensitivity of detection. The exception is cortisol, which is more water-soluble and therefore does pass into urine in significant amounts in its unconjugated form (“urinary free cortisol”) and can be measured directly by LC-MS/MS.

## Melatonin Metabolism

Melatonin is not a steroid hormone but has many of the same properties in that both are nonpolar, freely penetrate all tissues, and have a cellular nuclear receptor that binds and is activated by the ligand. Melatonin is synthesized from the amino acid tyrosine by the pineal gland in the brain in response to darkness. It freely diffuses into both the water soluble and nonpolar (membrane) parts of all tissues and cells. When passing through the liver much of the melatonin is hydroxylated at the 6-position, and then conjugated with sulfate to form its primary metabolite, 6-sulfatoxymelatonin (MT6s), and excreted into urine in preparation for elimination. MT6s is very stable in urine and its level in a urine void is reflective of the amount of active melatonin synthesis over a specific time frame. Thus, a first morning void is representative of peak melatonin synthesis that occurs at night during the dark phase.

## Dried Urine - A Convenient & Accurate Testing Option

The biggest drawback to liquid urine testing is sample collection. It is inconvenient to carry a urine jug around for 24 hours, and studies have also shown that as many as 40% of people make mistakes in accurately measuring urine volume, or missing some collections during the 24 hour period (e.g., during bowel movements).

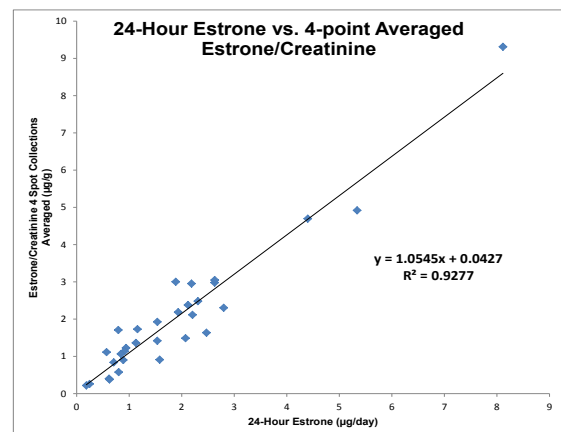
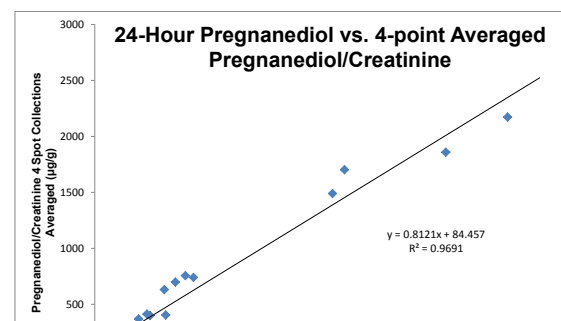
ZRT Laboratory has developed a convenient alternative to the cumbersome, inconvenient, and sometimes inaccurate 24-hour urine test. Instead of collecting all urine over a 24 hour period in one jug, the new test developed at ZRT includes the collection of four separate urine samples at key time points throughout the day: 1st morning, 2nd morning, early evening, and bedtime. Urine is collected on filter paper strip either by dipping it in urine collected in a cup, or by urinating directly on the strip. The urine-saturated filter strips are allowed to dry overnight and then sent to the laboratory for testing. The urine collection scheduling allows the patient to collect the samples at home and eliminates the inconvenience of carrying around a urine jug all day and having

to process the wet sample for shipment back to the testing laboratory. When results are averaged from the four samples the correlation to a 24-hour collection is excellent (see graphs showing the typical correlation for two common metabolites). For urinary free cortisol and melatonin assessment, results from each of the four samples are reported individually to give an appreciation of the circadian pattern of hormone production at different times throughout the day. This is not possible with a 24 hour collection.

Hormone and metabolite levels are expressed in  $\mu\text{g/g}$  of creatinine, measured in the same samples, to correct for differences in the hydration status of the patient.

## Estrogen Metabolism

Estrogens are essential for health and well being in both women and men. However, some oxidized estrogen metabolites have been shown to be harmful and increase risk for breast cancer in women, and prostate cancer in men. Cancer cells release these “bad” estrogen metabolites into the bloodstream where they are eventually eliminated in urine. Because urine has the highest concentration of these estrogen metabolites compared to any other body fluid, urine has been the fluid of choice to monitor the estrogen metabolites. The estrogens, estrone and estradiol, are metabolized primarily by two cytochrome P450 enzymes, cyp-1A1 and cyp-1B1. Cyp 1A1 converts E1 and E2 to 2-hydroxyestrogens, while Cyp 1B1 converts them to 4-hydroxyestrogens.





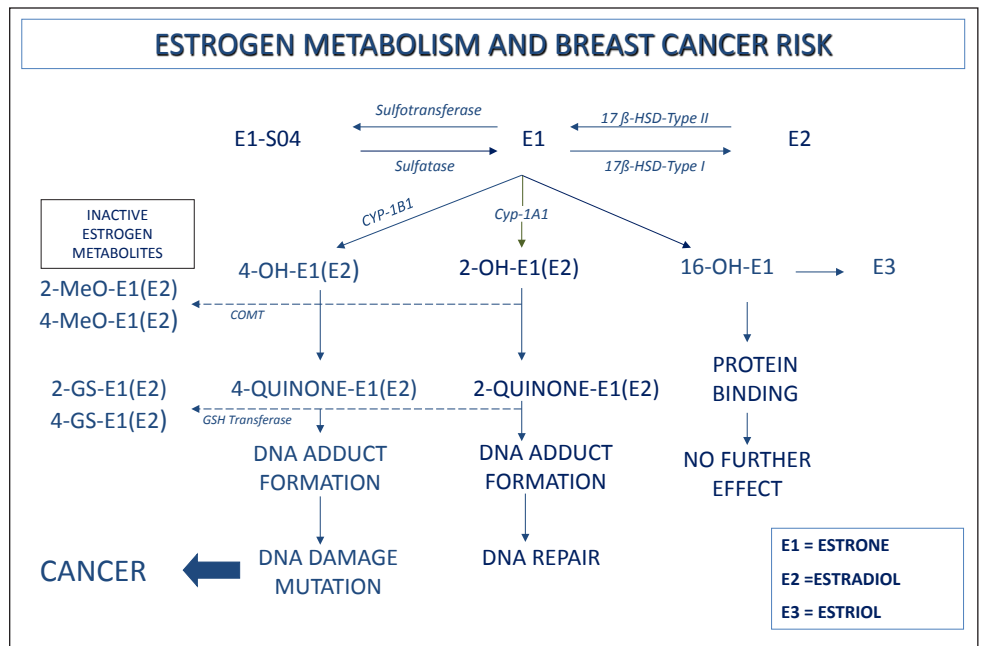
Both hydroxylated forms are known as catechol estrogens. The 2- or 4- hydroxylated estrogens are either methylated by Catechol Ortho Methyl Transferase (COMT) or oxidized to 2- or 4-estrogen quinones. Methylation of the hydroxyestrogens prevents their conversion to the more toxic estrogen quinones. The estrogen quinones are highly reactive molecules that form in the absence of anti-oxidants and the presence of oxidized lipids and heavy metals. If allowed to react with DNA the 4-quinone estrogens form covalent adducts that lead to deletion mutations in DNA and increase cancer risk. The 2-quinones form adducts but do not cause DNA mutations, and therefore are less harmful. In an unpublished clinical study with researchers in China, ZRT Laboratory has confirmed that urinary 4-hydroxyestrone and 4-hydroxyestradiol are significantly higher in women with active intact breast cancers than in a group of healthy women. Thus, by quantifying the amount of 2- and 4-hydroxyestrogens present in urine, as well as the capacity to detoxify the potentially dangerous 4-hydroxyestrogens by methylation, it is possible to evaluate breast cancer risk<sup>2</sup>.

The bad news may be that you are making too much of the 4-hydroxyestrogens and not methylating them well. The good news is that you know this is happening and can do things to change how your body metabolizes estrogens, and in doing so decrease your breast cancer risk. Nutritional intervention (e.g., foods rich in DIM or indole-3-carbinol)<sup>3</sup> can be used to shift estrogens in the direction of 2-hydroxylation, which is safer. Additionally, methylation of estrogen metabolites can be supported nutritionally (e.g., methyl donors such as vitamin B12, folic acid, SAM-e, and foods such as onions, garlic and beets)<sup>4</sup>. And the effectiveness of this intervention can be evaluated by measuring the ratio of 2- and 4-methoxyestrone to 2- and 4-hydroxyestrone.

## Progesterone Metabolism

In women the primary source of progesterone is ovarian. During the luteal phase of the menstrual cycle, progesterone is synthesized in the ovaries in large amounts and released into the bloodstream where it reaches target tissues throughout the body. Progesterone has a relatively short half-life in the bloodstream and is metabolized in the liver by various phase I and II enzymes. These metabolites are excreted by the kidneys into urine. Very little progesterone itself enters urine, whereas progesterone metabolites are readily excreted in urine. One

of the primary progesterone metabolites is a glucuronide conjugate of pregnanediol. Levels of this progesterone metabolite are very similar to active levels of progesterone in the bloodstream, making pregnanediol a convenient surrogate marker of progesterone synthesis. About half of progesterone is metabolized in the liver (hepatic metabolism) and the other half in other organs or tissues (extrahepatically)<sup>5</sup>. Hepatic metabolism is usually about 50% by 5 $\alpha$ -reductase and 50% by 5 $\beta$ -reductase. In total 60-70% follows the 5 $\alpha$  pathway and only 25% the 5 $\beta$  pathway. The 5 $\alpha$  pathway creates allopregnanolone, which enters the brain and binds to GABA receptors and has a calming, sleep-inducing action<sup>6</sup>. Progesterone used as a supplement raises both circulating and urine levels of progesterone and its metabolites. Topical progesterone has little effect on urinary levels



of progesterone metabolites since little of it enters the liver or kidneys. Oral progesterone, on the other hand, passes through the liver where it is extensively converted to metabolites that are excreted by the kidneys into urine.

Other than pregnanediol, the principle metabolite of progesterone, two other categories of progesterone metabolites are present in urine, pregnane and pregnene metabolites. Allopregnanolone belongs to the pregnane class. Cancer researchers have found that the pregnane category of metabolites has growth-promoting effects on cancer cells, while the pregnene category has the opposite, i.e., growth-inhibitory actions. Therefore, knowing the amount of progesterone present, determined by the surrogate pregnanediol, as well as the distribution of pregnane and pregnene metabolites may help determine if progesterone could be helpful or harmful as regards breast cancer risk.



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# Hormone Metabolite Testing

## Non-invasive home test kit

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This could be an important distinction in women at risk for breast cancer, who may need to be advised against progesterone supplementation if they show higher levels of the pregnane metabolites relative to the pregnene metabolites<sup>7</sup>.

**NOTE:** Topical application of progesterone and other steroids does not increase urinary levels of progesterone or its metabolites to any significant extent, but results in dose-dependent increases in salivary and capillary blood progesterone levels, as well as tissue uptake and clinical response. Therefore, urinary testing should not be used to evaluate the effectiveness of topically delivered progesterone.

### Androgen Metabolism

Androgens play an important role in health and well-being. Testosterone is synthesized by the ovaries, testes, and adrenal glands from the precursors DHEA and androstenedione, and converted to the more potent androgen, 5 $\alpha$ -DHT directly in target tissues by 5-alpha reductase, or to estradiol via aromatase. Two androgen precursors, DHEA and androstenedione, and six downstream androgens, testosterone, Epi-testosterone (Epi-T), 5 $\alpha$ -DHT, etiocholanolone, androsterone, and 5 $\alpha$ ,3 $\alpha$ -androstanediol are tested. Testing for androgens in urine reveals potential excesses or deficiencies of the primary androgen precursors DHEA and androstenedione, and provides information about the extent of their conversion to their down-stream metabolites. Epi-T and testosterone are produced in near equal amounts from DHEA/ androstenedione; however, Epi-T is metabolically inert. Epi-T is well known in the context of sports doping. While typically found in a 1:1 ratio with testosterone, individuals taking exogenous testosterone have a high T/Epi-T ratio, which usually rises above 6. The T/Epi-T ratio can also be helpful in interpreting cases with testosterone supplementation.

An excess of 5 $\alpha$ -DHT contributes to hair loss, acne, and hirsutism in men and women and increased risk for BPH and prostate cancer in men (but only when associated with high estrogens). While this highly androgenic metabolite is of great interest

and importance, it is largely created intracellularly and quickly metabolized to 3 $\alpha$ -androstanediol. 5 $\alpha$ -reductase inhibitors like finasteride and dutasteride are commonly prescribed to reduce the production of 5 $\alpha$ -DHT; however, herbs and certain supplements, e.g., saw palmetto, are also used<sup>8</sup>. Hormones like progesterone that compete with 5 $\alpha$ -reductase are also used to reduce 5 $\alpha$ -DHT formation; however, there is growing concern that the 5 $\alpha$  progesterone metabolites (pregnane metabolites) might pose a greater risk for breast tumor growth (see above).

Androstenedione is metabolized to estrone and testosterone is metabolized to estradiol via the enzyme aromatase. In men, high aromatase activity can contribute to gynecomastia and testosterone deficiency symptoms. In women, aromatase has been implicated in the etiology of breast cancer<sup>9</sup>. Aromatase inhibitors are commonly prescribed to suppress conversion of testosterone to estrogens. Recent studies have shown lower breast cancer risk in women using androgens in combination with aromatase inhibitors<sup>10</sup>.

### Cortisol Metabolism

The four-point diurnal free cortisol and cortisone test included in the Adrenal Metabolites profile, as well as the Advanced Metabolites profile, gives insight into the impact of stressors on adrenal function. The diurnal pattern of free cortisol in urine mirrors saliva cortisol, as both reflect the non-protein-bound bioavailable fraction.

Total cortisol production and cortisol metabolism can also be ascertained from urine testing in the Advanced Metabolites profile. This creates valuable insight into what is happening at the tissue level. These profiles assess total daily cortisol and cortisone production, measuring both free and conjugated hormone over all four daily samples, and levels of their primary metabolites tetrahydrocortisol (THF) and tetrahydrocortisone (THE).

Cortisol is reversibly metabolized to cortisone in tissues. Relative amounts of cortisol and cortisone are determined by

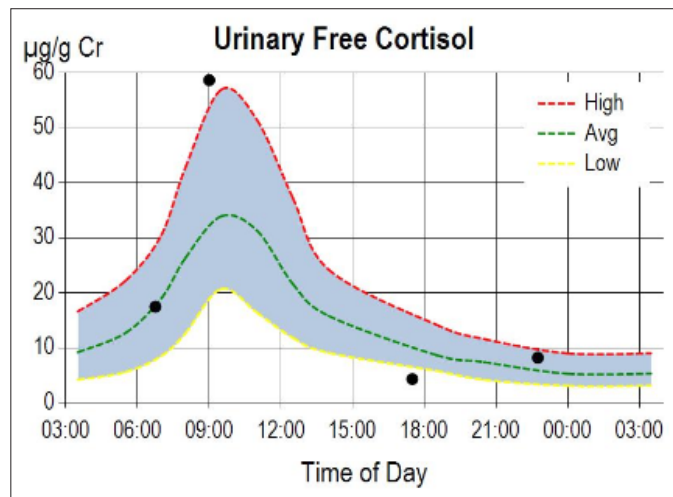
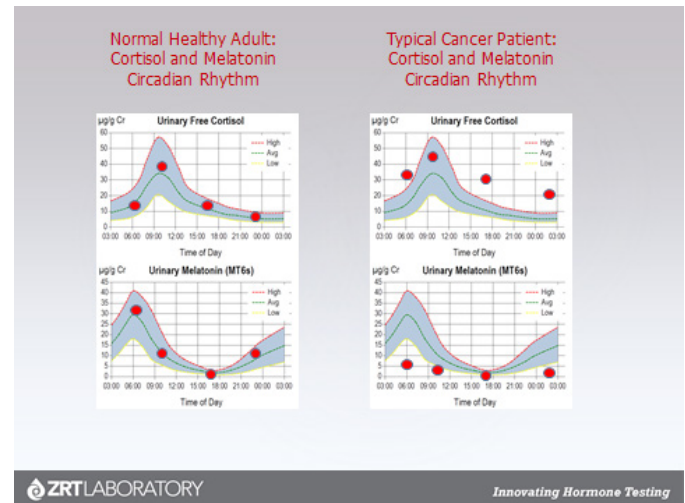
the relative activity of 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ HSD): 11 $\beta$ HSD type 1 converts inactive cortisone to cortisol, while 11 $\beta$ HSD type 2 converts cortisol to cortisone, effectively suppressing cortisol activity. 11 $\beta$ HSD1 predominates in liver, adipose tissue, gonads, brain, vascular smooth muscle, and skeletal muscle; 11 $\beta$ HSD2 predominates in the kidney, colon, GI tract, and salivary glands.

Cortisol is irreversibly metabolized for disposal in the liver to dihydrocortisol and then tetrahydrocortisol (THF); there is a corresponding pathway for disposal of cortisone to form tetrahydrocortisone (THE). Liver metabolism to THF and THE is catalyzed by 5 $\alpha$  and 5 $\beta$  reductases, therefore elevations in the activity of these enzymes will increase cortisol metabolism and levels of THF and THE. Increased cortisol metabolism in turn drives increased cortisol production from the adrenal glands via the HPA axis, which stimulates ACTH (adrenocorticotropic hormone) secretion by the pituitary gland. This has the side effect of also increasing adrenal androgen production, as seen for example in PCOS when insulin resistance results in increased 5 $\alpha$ -reductase activity. The ratio of THF to THE reflects the tissue cortisol/cortisone ratio. A high THF/THE ratio is seen in essential hypertension, while a low ratio is seen in insulin resistance and obesity<sup>11</sup>. Cortisol metabolite levels are also affected by the increased and decreased metabolism of cortisol seen in hyper- and hypothyroidism<sup>12</sup>.

hormones or supplements. Graphing of the free cortisol and free cortisone results showing actual test times is a unique feature of ZRT test reporting.

## Mineralocorticoids

The Advanced Metabolites profile includes the progesterone metabolites deoxycorticosterone (DOC) and corticosterone, which are aldosterone precursors. DOC is a weak mineralocorticoid itself and very high levels of progesterone therapy may lead to high DOC levels. These metabolites may give insight into aldosterone formation as it relates to sodium and potassium levels, water retention, and blood pressure.



The graph above is an example of the free cortisol graph shown on a patient report for the Adrenal and Advanced Metabolites profiles, giving the four point values as they relate to reference range values. The report also includes a graph of the free cortisone results. In this example, the patient shows a normal diurnal pattern with higher mid-morning levels after the first morning nadir, and levels dropping towards evening and night. The slightly lower evening level reflects lower adrenal output, possibly due to the effects of stress or the use of exogenous

## Melatonin

Melatonin, a hormone produced by the pineal gland during the dark phase of the light/dark cycle, regulates the sleep/wake cycle and the “biological clock.” However, it is known also to have a potent anticancer effect, particularly in hormone-dependent cancers such as breast and prostate cancers. It inhibits tumor growth through a variety of mechanisms<sup>13</sup>, including antioxidation, antiestrogenic actions<sup>14</sup>, promotion of apoptosis, and immune system activation. Melatonin acts as a selective estrogen receptor modulator in breast tumor cells and also down-regulates aromatase, reducing local estrogen synthesis from androgenic precursors<sup>15</sup>. Low night time melatonin levels are seen in breast<sup>16</sup> and prostate cancer<sup>17</sup> patients; the pineal gland which secretes melatonin is more likely to be calcified in advanced breast cancer patients. The WHO’s International Agency for Research on Cancer has concluded that “shift work that involves circadian disruption is probably carcinogenic to humans,” probably because of the suppression of melatonin production by exposure to light during the night<sup>18</sup>. The graphs above are examples of the graph shown on a patient report, giving the four point values as they relate to reference range values for melatonin and cortisol. Melatonin production is

greatest during the night-time hours, resulting in a peak urinary MT6s early in the morning. Melatonin is gradually suppressed during daylight hours and rises during the evening towards bedtime. Compared to a normal healthy adult, a typical breast cancer patient shows suppressed melatonin production.

Diurnal MT6s is included in the Advanced Metabolites profile.

## Bisphenol A

Bisphenol A (BPA) is a ubiquitous environmental endocrine disruptor; a CDC study found detectable levels in 95% of urine samples tested<sup>19</sup>. BPA is used in the production of polycarbonate plastics and epoxy resins, and is found principally in linings of food and beverage cans and in thermal paper used in receipts. Exposure occurs mainly from consuming canned foods and beverages, and absorption via the skin when handling cash register receipts or paper money, which has been found to be contaminated with BPA through incidental contact with receipts. Clearance of BPA from the body is significantly less efficient in infants and the developing fetus than in adults, leading to a greater buildup of levels and increased susceptibility to its endocrine-disrupting effects. It is therefore particularly important to limit BPA exposure during pregnancy or breastfeeding or in early childhood. BPA's endocrine-disrupting effects are mediated through its strong binding to estrogen-related receptor  $\gamma$  (ERR- $\gamma$ ) and, with lower affinity, to estrogen receptor (ER)  $\alpha$  and  $\beta$ .

Higher BPA exposure has been linked with increased risk for coronary heart disease<sup>20,21</sup> and prostate cancer<sup>22</sup>. It has been found in experimental studies to induce insulin resistance<sup>23</sup> and disrupt thyroid hormone activity<sup>24</sup>. Exposure in fetuses and/or young children disrupts secondary sexual and neuro-behavioral development<sup>25</sup> and increases incidence of childhood asthma<sup>26</sup>. Early life exposure can also cause long-term health issues via epigenetic mechanisms; these include increased risk of hormone-dependent cancers (e.g., breast and prostate cancer), infertility, and pancreatic function abnormalities<sup>27</sup>. BPA's effects on estrogen-receptor-mediated transcriptional processes are additive to those of soy phytoestrogens, and so the use of soy formula in infants also exposed to BPA may produce estrogenic effects that further enhance the risk of breast cancer in later life<sup>28</sup>.

BPA testing is included in the Estrogen Elite and Advanced Metabolites profiles.

## Advantages of Dried Urine for Testing Hormone Metabolites

- ▶ Collecting four dried urine strips is easier and more convenient than a 24-hour urine collection because it does not require handling, measurement, or freezing of liquid urine samples
- ▶ Four spot collections during the day instead of a full 24-hour collection takes away the stress of having to collect every single urine void and carrying around a urine jug all day long
- ▶ "Free" cortisol and cortisone measurements at 4 time points, along with cortisol metabolite ratios, provides the most comprehensive cortisol profile available
- ▶ First morning MT6s represents night-time melatonin production – no need to sample in the middle of the night, disturbing normal melatonin production during the dark cycle and therefore improving clinical accuracy
- ▶ Hormone metabolites are exceptionally stable in dried urine for weeks at room temperature, allowing more flexibility in collection, shipment, testing, and storage than liquid urine collections
- ▶ Results expressed in  $\mu\text{g/g}$  creatinine take into account the hydration status of the patient, so that test results are accurate even when urine is very concentrated or dilute

## Considerations

- ▶ Because the urine test results are given relative to creatinine, the test assumes proper kidney function, and therefore should not be used for individuals with compromised renal function.
- ▶ The relationship to creatinine can also be compromised if a sample is excessively dilute. Because of this, testing individuals must restrict their fluid levels to normal consumption during the day of testing, particularly in the two hour window preceding each collection.
- ▶ Oral hormone supplementation generates a much higher level of metabolites in urine that are due to the first-pass effect (gut and liver metabolism). Because of this, metabolite ratios can still be properly interpreted but absolute levels of hormones like estradiol will likely represent an overestimation of the body's bioavailable fraction of supplemented hormone. Saliva testing is more likely to provide a more accurate representation of the bioavailable fraction of hormone delivered orally.
- ▶ Vaginal hormones should be monitored with great care as the hormones may directly contaminate the urine sample and lead to false-high results. This is particularly true for vaginal estrogens and androgens. Avoid vaginal delivery of hormones for at least three days prior to urine collection.



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