



SLEEP BALANCE PROFILE

DIURNAL MELATONIN/CORTISOL IN DRIED URINE

Sleep Balance Profile

Tests included in the Profile:

Melatonin (MT6s x 4)

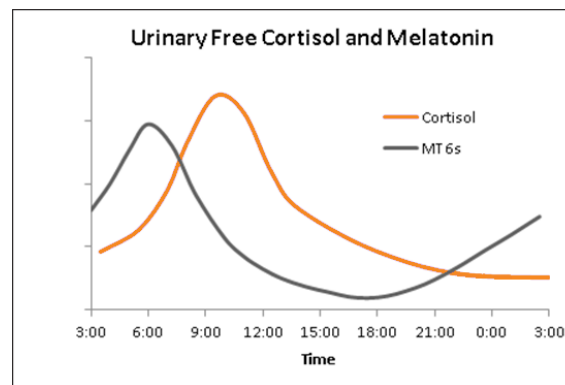
Free Cortisol (C x 4)

A Simple, Convenient, At-Home Testing Option

- Circumvents the disadvantages of serum, saliva, and 24-hour urine tests commonly used for testing these hormones
- Collecting four dried urine strips at four time points during the day is easy and convenient
- First morning MT6s represents night time melatonin production - no need to sample in the middle of the night
- Cortisol and MT6s are exceptionally stable in dried urine for weeks at room temperature, allowing flexibility in collection, shipment, testing, and storage
- 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

Adequate sleep has long been known to be vital to good health. 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 now known also to have free radical scavenging and antioxidant properties and a significant role in stimulating the immune system to protect against the growth of abnormal tissues such as breast and prostate cancers. Melatonin is also neuroprotective. Adequate melatonin production during the night, as well as suppression of production during the day by exposure to daylight, form a balance that is vital to optimal health. Circulating melatonin is rapidly and efficiently hydroxylated and conjugated with sulfate in the liver to form its primary metabolite, 6-sulfatoxymelatonin (MT6s), and excreted into urine; it is this metabolite that is measured in the Sleep Balance Profile.

Cortisol, a hormone produced by the adrenal glands in response to stress, is also known for its diurnal variation linked to the sleep/wake cycle. It has the opposite pattern to melatonin production in a healthy individual. While melatonin rises at night to peak during the early hours of the morning, cortisol is at its lowest levels throughout the night. After waking, melatonin production dips with the onset of daylight and cortisol rises and peaks about 30 minutes to 1 hour after rising. Cortisol production falls gradually during the day, while melatonin begins to rise during the evening as daylight diminishes, and the cycle repeats itself. When the cortisol pattern is disrupted, for example as a result of excessive stressors, this can lead to high night cortisol, disrupted sleeping patterns, and exposure to more night time light, which in turn leads to lowered levels of melatonin. Thus, excessive stressors can lead to higher cortisol and indirectly lower melatonin synthesis, preventing melatonin from carrying out its other beneficial and protective functions.



The graph shows the typical circadian variation in urinary melatonin and cortisol levels (ZRT data). 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. In an opposing pattern, night-time cortisol production is at its lowest; it is normally at its highest immediately after waking, peaking in the urine around 2 hours later. Cortisol levels drop towards the end of the day, with progressively lower levels in the evening as the night-time nadir is approached.

Dried Urine Testing.

Non-invasive home test kit.

Cortisol and Melatonin Imbalance and Sleep Disturbance

Lack of sleep can be detrimental to health on a number of levels. Sleeplessness at night results in lack of alertness during the day, impairing judgment and increasing risk of accidents. Chronic sleeplessness affects skin appearance and health, reduces libido and overall vitality, reduces cognitive function, contributes to weight gain, and increases risk of diabetes and cardiovascular diseases.

The diurnal cortisol pattern reflects hypothalamic-pituitary-adrenal (HPA) axis function, with levels of cortisol normally highest immediately after waking and dropping throughout the day to a night-time nadir. Abnormal patterns include elevated cortisol levels throughout the day and night in chronic stress, a suppression of cortisol levels in the condition known as adrenal fatigue, and a recognizable diurnal variation, but with overall lower cortisol levels at each time point in chronic fatigue syndrome¹.

Chronic insomnia is closely related to stress. A disrupted diurnal cortisol pattern with high night-time levels can be an indicator of a stress response that extends into the night-time hours, causing hyperarousal that disrupts sleep. Conversely, deep sleep suppresses the HPA axis and keeps cortisol low². Insomnia related to stress can be improved by lifestyle modifications and therapies to reduce stress.

Because of its established role in the regulation of the circadian rhythm, treatment with exogenous melatonin has been found useful in people with circadian rhythm sleep disorders, such as delayed sleep phase disorder, jet lag, shift worker disorder, and the non-24-hour sleep-wake disorder most commonly found in totally blind individuals; however, its utility for the treatment of insomnia is not established and remains controversial³.

Impact of Chronic Sleep Disruption on Diseases of Aging (Cancer, Diabetes, Alzheimer's Disease)

Chronic sleep problems resulting in longer-term HPA axis dysregulation and deficiencies in melatonin production can impact multiple aspects of overall health. Melatonin is a potent free radical scavenger and stimulates the release of antioxidative enzymes, thereby protecting DNA and other molecules from injury^{4,5}. Melatonin's ability to prevent oxidative stress is demonstrated in placebo-controlled studies⁶; it also protects against diseases related to premature aging, as well as cancer and cardiovascular disease.

Cancer

Melatonin has a potent anticancer effect, particularly in hormone-dependent cancers. It inhibits tumor growth through a variety of mechanisms⁷, including antioxidation, antiestrogenic actions⁸, 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 precursors⁹. Low night time melatonin levels are seen in breast¹⁰ and prostate cancer¹¹ 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 night¹².

Insulin Resistance/Type 2 Diabetes

Sleep disturbances, including obstructive sleep apnea, sleep deprivation, and shift work, contribute to the development of insulin resistance and the metabolic syndrome¹³. While chronic HPA axis hyperactivity, typified by higher than normal night-time cortisol, contributes to insomnia, it is also thought to be a consequence of obstructive sleep apnea¹⁴, because nocturnal awakenings result in pulsatile cortisol release. HPA axis activation due to sleep apnea can exacerbate glucose intolerance in metabolic syndrome and lead to diabetes, with

consequent increased cardiovascular disease risk.

A low nocturnal melatonin is also associated with insulin resistance and high insulin levels¹⁵, and women with low nocturnal melatonin have been found to be more likely to develop type 2 diabetes¹⁶. Rat studies have indicated a mechanism for the link between melatonin and obesity; melatonin increases leptin, a hormone that regulates appetite, in the presence of insulin in rat adipocytes¹⁷, and melatonin was also found to be involved in lipid metabolism, enhancing fat mobilization and reducing fat storage in response to exercise training¹⁸. This could have implications for susceptibility to obesity in people with low melatonin as a result of shift work or insomnia.

Brain Health - Alzheimer's Disease

Age-associated neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's Diseases have been linked with oxidative damage; melatonin levels are known to decline with advancing age, and this is thought to contribute to the rise in oxidative stress that may contribute to these disorders¹⁹. A decline in nocturnal melatonin production is common in patients with Alzheimer's disease, and sleep-wake disturbances increase as the disease progresses. The use of melatonin and its analogs for the treatment or prevention of Alzheimer's disease is under investigation.

Methods to Assess Melatonin and Cortisol Balance

An abundance of scientific literature supports the use of serum/plasma, saliva, and 24-hour urine to assess the levels of melatonin and cortisol in humans. Each has advantages and disadvantages. Serum/plasma testing has the disadvantage that it requires venipuncture at times when melatonin is peaking (e.g., 2:00 am), and total cortisol is often inaccurate due to use of hormones (e.g. estrogens) that increase cortisol binding globulin, which lower actual bioavailable cortisol. Saliva has the advantage that samples can be collected by the individual at multiple times during the day, and the melatonin and cortisol levels represent the bioavailable fraction of these hormones. However, the disadvantage of saliva testing to assess the sufficiency of melatonin production during the night is that it requires collecting a sample at 2 a.m. when melatonin normally peaks, which

is inconvenient, and the annoyance of having to get up after attempting to sleep for 2-4 hrs may affect melatonin levels. Serum and 24-hour urine have the disadvantages that they only represent the average melatonin and cortisol production throughout the day, and don't provide an appreciation for the opposing diurnal patterns of these two hormones.

ZRT Laboratory has developed a melatonin and cortisol dried urine test that circumvents the disadvantages of serum, saliva, and 24-hour urine tests commonly used for testing these hormones. Urine is collected on filter strips at 4 time points throughout the day that are representative of the peaks and troughs of melatonin and cortisol production. The advantage of the dried urine test is that the first urine void represents the 8 hours or so of overnight peak melatonin production, a time when melatonin should be high and cortisol low. Melatonin is measured as the sulfated conjugate of its primary metabolite, known as MT6s, which is extremely stable in urine that collects in the bladder overnight, and on the filter strips sampled from the first morning void. The second urine sample, which is collected about 2 hours later, reflects the cortisol awakening response, when the melatonin should be falling rapidly from the first overnight level, and cortisol should be peaking. Urine is collected in the evening when melatonin and cortisol should both be low, and then again just before bed when the melatonin should be rising and cortisol at its nadir.

References

1. Jerjes WK, Peters TJ, Taylor NF, et al. Diurnal excretion of urinary cortisol, cortisone, and cortisol metabolites in chronic fatigue syndrome. *J Psychosom Res.* 2006;60(2):145-53.
2. Basta M, Chrousos GP, Vela-Bueno A, Vgontzas AN. Chronic Insomnia and the Stress System. *Sleep Medicine Clinics* 2007;2(2):279-91.
3. Vela-Bueno A, Olavarrieta-Bernardino S, Fernández-Mendoza J, Aguirre-Berrocal A. Melatonin, Sleep, and Sleep Disorders. *Sleep Medicine Clinics* 2007;2(2): 303-12.
4. Reiter RJ. Melatonin: clinical relevance. *Best Pract Res Clin Endocrinol Metab.* 2003;17(2):273-85.
5. Reiter RJ, Tan DX, Gitto E, et al. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Pol J Pharmacol.* 2004;56(2):159-70.
6. Herrera J, Nava M, Romero F, Rodríguez-Isturbe B. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration. *Am J Kidney Dis.* 2001;37(4):750-7.
7. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, et al. Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem.* 2010;17(36):4462-81.
8. Sánchez-Barceló EJ, Cos S, Fernández R, Mediavilla MD. Melatonin and mammary cancer: a short review. *Endocr Relat Cancer.* 2003 Jun;10(2):153-9.
9. Cos S, González A, Martínez-Campa C, et al. Estrogen-signaling pathway: a link between breast cancer and melatonin oncostatic actions. *Cancer Detect Prev.* 2006;30(2):118-28.
10. Schernhammer ES, Berrino F, Krogh V, et al. Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst.* 2008;100(12):898-905.
11. Sigurdardottir LG, Markt SC, Rider JR, et al. Urinary melatonin levels, sleep disruption, and risk of prostate cancer in elderly men. *Eur Urol.* 2014 Aug 5.
12. Straif K, Baan R, Grosse Y, et al. Carcinogenicity of shift-work, painting, and fire-fighting. *Lancet Oncol.* 2007;8(12):1065-6.
13. Wolk R, Somers VK. Sleep and the metabolic syndrome. *Exp Physiol.* 2007;92(1):67-78.
14. Buckley TM, Schatzberg AF. On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. *J Clin Endocrinol Metab.* 2005;90(5):3106-14.
15. McMullan CJ, Curhan GC, Schernhammer ES, Forman JP. Association of nocturnal melatonin secretion with insulinresistance in nondiabetic young women. *Am J Epidemiol.* 2013;178(2):231-8.
16. McMullan CJ, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. *JAMA.* 2013;309(13):1388-96.
17. Alonso-Vale MI, Andreotti S, Peres SB, et al. Melatonin enhances leptin expression by rat adipocytes in the presence of insulin. *Am J Physiol Endocrinol Metab.* 2005;288(4):E805-12.
18. Borges-Silva CN, Fonseca-Alaniz MH, Alonso-Vale MI, et al. Reduced lipolysis and increased lipogenesis in adipose tissue from pinealectomized rats adapted to training. *J Pineal Res.* 2005;39(2):178-84.
19. Srinivasan V, Pandi-Perumal SR, Cardinali DP, Poeggeler B, Hardeland R. Melatonin in Alzheimer's disease and other neurodegenerative disorders. *Behav Brain Funct.* 2006;2:15.