

Urinary Neurotransmitter Analysis

The following includes summaries of studies that used urinary neurotransmitter analysis as a biomarker for the examination of various disorders including depression, anxiety and post-traumatic stress disorder.

- 1. Hughes JW, Watkins L, Blumenthal JA, Kuhn C, Sherwood A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J Psychosom Res* 2004; 57(4): 353-8.**

In this study conducted by Hughes et al at the Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, the researchers examined the relationship between depression and anxiety and urinary neurotransmitter and cortisol excretion. Ninety-one women were evaluated for depression (using the Beck Depression Inventory) and anxiety (using the Spielberger State-Trait Anxiety Inventory). Twenty-four hour urine collections were assayed for each participant measuring norepinephrine, epinephrine and cortisol production. The results of this study showed that higher levels of depressed symptoms in women were associated with higher levels of urinary norepinephrine excretion. Higher levels of anxiety were associated with higher urinary norepinephrine and cortisol excretion. This research suggests that exacerbated symptoms of depression and anxiety may be associated with increased sympathetic nervous system (SNS) activity. These results are consistent with the possibility that increased SNS activity may play a role in increased mortality associated depression in older adults.

- 2. Yehuda R, Southwick S, Giller EL, Ma X Mason JW. Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *J Nerv Ment Dis* 1992; 180(5): 321-5.**

In this study, conducted at the Department of Psychiatry, Mount Sinai Medical School by Yehuda et al, urinary measurements of the neurotransmitters dopamine, norepinephrine and epinephrine were used as biomarkers to measure the severity of posttraumatic stress disorder (PTSD) in Vietnam combat veterans. Twenty-two male patients (14 inpatients and eight outpatients) with PTSD, as well as 16 nonpsychiatric normal males participated in this study. This study found that urinary dopamine and norepinephrine levels were significantly correlated with the severity of PTSD symptoms. The researchers concluded that these findings supported the theory that enhanced sympathetic nervous system (SNS) activation plays a major role in

PTSD and that increased SNS arousal may be closely linked to the severity of certain PTSD clusters.

- 3. Hernandez-Reif M, Ironson G, Field T, Hurley J, et al. Breast cancer patients have improved immune and neuroendocrine functions following massage therapy. *J Psychosom Res.* 2004; 57(1): 45-52.**

This study was conducted at the University of Miami School of Medicine by Hernandez-Reif et al. Cancer patients are at an increased risk of suffering from depression and anxiety. Depression and anxiety can compromise immunological function in Cancer patients, including natural killer (NK) cell activity. Stress has been linked to increased tumor development by decreasing NK cell activity. In this study, the researchers examined the use of massage therapy as a treatment option for stress reduction and mood enhancement using urinary neurotransmitter measurements (Norepinephrine, Epinephrine, Dopamine and Serotonin) as biological markers. Blood measurements were also drawn to study the effects of stress reduction and mood on the immune system. Thirty- four women diagnosed with Stage 1 or 2 breast cancer were randomly assigned to the massage therapy or a control group. The massage therapy group received 30 minute massages three times per week for 5 weeks. **The long-term massage effects included a reduction in depression and hostility along with increased urinary dopamine and serotonin values, NK cell number, and lymphocytes.** The researchers concluded that massage therapy for cancer patients may provide benefits for mood enhancement and immune system support.

- 4. Delahanty DL, Nugent NR, Christopher NC, Walsh M. Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendocrinology* 2005; (2): 121-8.**

Previous research has suggested that children with posttraumatic stress disorder (PTSD) have altered levels of catecholamines (Dopamine, Norepinephrine, and Epinephrine) as compared to children that have suffered from trauma that do not have PTSD. The researchers in this study want to again examine whether a significant variance in urinary cortisol and neurotransmitter excretion following a traumatic event in children may be associated with an increased risk for the development of PTSD. Urinary catecholamine and cortisol measurements were conducted on 82 children aged 8-18 that were admitted to a Level 1 trauma center. The urine samples were immediately collected upon admission. Additional assessments included PTSD and depressive symptomatology for 6 weeks following the initial traumatic event. **The results of this study indicated that elevated initial urinary cortisol and epinephrine levels immediately following a traumatic event continued to predict the development of acute PTSD symptoms, particularly in boys.**

5. **Tripodianakis J, Markianos M, Sarantidis D, Agouridaki M. Biogenic amine turnover and serum cholesterol in suicide attempt. Eur Arch Psychiatry Clin Neurosci 2002; 252(1): 38-43.**

In this study, the researchers investigated the correlation between suicidal behavior and changes in neuronal activity. The researchers examined the urinary neurotransmitter turnover in one hundred eleven subjects that were admitted to a hospital following suicide attempt. The urine metabolites of the neurotransmitters serotonin, dopamine and norepinephrine (5-HIAA, HVA, MHPG respectively) were collected within 24 hours of admission. These urine samples were compared to urine neurotransmitter metabolite turnover in a group of 62 healthy controls. According to psychiatric diagnosis made, according to DSM-III-R criteria, 54 subjects in the suicide attempt group were diagnosed with adjustment disorder, 25 were diagnosed with depression, 16 with schizophrenia, and 16 with personality disorder. Within all subgroups of patients diagnosed with various disorders following suicide attempt, a significant increase in the urinary norepinephrine metabolite MHPG was found, versus normal controls.

6. **Lykouras L, Markianos, Hatzimanolis J, Malliaras D, Stefanis C. Association of biogenic amine metabolites with symptomatology in delusional (psychotic) nondelusional depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 1995; 19(5): 877-887.**

In this study, the urine metabolites of the neurotransmitters norepinephrine, serotonin, and dopamine (MHPG, 5HIAA and HVA respectively) were examined in 84 patients diagnosed with major depressive disorder. Fifty of the 84 patients were nondelusional, 34 were diagnosed delusional (psychotic) per DSM-III-R criteria. In the delusional group, norepinephrine metabolite excretion was positively related to scores of depressed mood and insomnia. Serotonin metabolite excretion was negatively associated with insomnia, work and interests. In both the delusional and nondelusional groups Dopamine metabolite was positively related to agitation.

7. **Takahashi S, Takahashi R, Masumura I, Miike A. Measurement of 5-hydroxyindole compounds during L- 5-HTP treatment in depressed patients. Folia Psychiatr Neurol Jpn 1976; 30(4): 461-73.**

In an open clinical trial, 5-hydroxytryptophan (5-HTP), an immediate precursor to serotonin was given to hospitalized patients suffering from depression. The patients received 150 mg of 5-HTP for seven days. Seven of 14 patients (50%) responded to the small dose of 5-HTP with mild to moderate improvement of their depression. Urinary excretion levels and plasma concentrations of three 5-hydroxyindole compounds, 5-HTP, 5-HT and 5-HIAA, were measured during the treatment. In this study, the researchers found that patients that did not have positive improvement in their symptoms of depression, following oral treatment with 5-HTP, exhibited significantly lower excretion levels of the serotonin metabolite 5-HIAA in urine. The researchers concluded that 5-

HTP may not have been fully utilized in the depressed patients who did not react positively to the agent.

8. Giulianoa François, Allardb J. Dopamine and Male Sexual Function. *European Urology* 2001;40:601-608.

The use of the D1/D2 dopamine receptor agonist apomorphine for the treatment of erectile dysfunction provides strong support in favor of a participation of the dopaminergic system in the control of sexual function. However, the exact involvement of dopamine in the control of sexual motivation and genital arousal in males is unknown. Experimental data in male rats suggested an implication of dopamine in sexual motivation as well as in copulatory performance. Specific tests allowing assessment of sexual motivation showed that the release of dopamine at the level of the nucleus accumbens (innervated by the mesolimbic dopaminergic pathway) and the medial preoptic area of the hypothalamus (innervated by the dopaminergic incertohypothalamic pathway) positively regulated the anticipatory/motivational phase of copulatory behavior. A permissive role of dopamine released at the level of the median preoptic area of the hypothalamus in the display of copulatory behavior has also been demonstrated. It is noteworthy that these participations of the dopaminergic system are not specific for sexual behavior but rather reflect the involvement of dopamine in the regulation of cognitive, integrative and reward processes. Because of its role in the control of locomotor activity, the integrity of the nigrostriatal dopaminergic pathway is also essential for the display of copulatory behavior. Somehow more specific to sexual function, it is likely that dopamine can trigger penile erection by acting on oxytocinergic neurons located in the paraventricular nucleus of the hypothalamus, and perhaps on the proerectile sacral parasympathetic nucleus within the spinal cord. In conclusion, central dopamine is a key neurotransmitter in the control of sexual function.

9. Roach, J. Gamma-aminobutyric acid inhibits synergistic interleukin-6 release but not transcriptional activation in astrocytoma cells. *Neuroimmunomodulation* . Volume: 15, Issue: 2, Date: 2008 , Pages: 117-24, 2008.

A decline in the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) may enhance cytokine release in Alzheimer's disease (AD) resulting in neuroinflammation. We investigated the GABA-mediated suppression of the synergistic release of interleukin (IL)-6 due to interleukin 1-beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha). METHODS: Rat C6 astrocytoma cells were treated with IL-1 beta and TNF-alpha in the absence and presence of GABA. Activation of p38, degradation of I kappaB-alpha and total cellular IL-6 were determined by Western blot analysis. IL-6 release and gene expression were measured by ELISA and RT-PCR, respectively. RESULTS: Although p38 and nuclear factor (NF)-kappaB are essential for the synergistic release of IL-6, GABA did not affect either p38 phosphorylation or I kappaB-alpha degradation. Additionally, GABA suppressed IL-6 release but did not alter cytokine-driven synergistic increases in IL-6 gene expression. Western blot analysis revealed that co-treatments with IL-1 beta and TNF-alpha resulted in an increase in intracellular IL-6 that was prevented by GABA. CONCLUSION: GABA-induced inhibition of IL-6 release appears to coincide with a reduction in cellular IL-6. The GABA-induced suppression of IL-6 release may include inhibition of IL-6 gene translation.

10. Marit A.C. Tanke a,b, et al. Low plasma tryptophan in carcinoid patients is associated with increased urinary cortisol excretion. *Psychoneuroendocrinology* (2008) 33, 1297—1301.

Background: Previously we observed in patients suffering from a metastatic carcinoid tumor that irritability, aggression and lack of impulse control are associated with low levels of plasma tryptophan and presumably with low brain serotonin function. In rats we showed that a diet of low tryptophan resulted in higher stress responses and higher corticosterone production. Here we tested in carcinoid patients whether tryptophan depletion due to tumor 5-HT overproduction is associated with high cortisol production.

Methods: Urinary excretion of cortisol, serotonin, 5-hydroxyindole acetic acid (the main metabolite of serotonin a marker of tumor activity), plasma levels of tryptophan and platelet content of serotonin (index of peripheral serotonin synthesis) were determined in metastatic midgut carcinoid patients. Patients (N = 25) were divided into two groups based on their plasma tryptophan levels (<25 mmol/l, n = 12 and >49 mmol/l, n = 13).

Results: Carcinoid patients with low plasma tryptophan levels had significantly higher urinary excretion of free cortisol (p < 0.01), independent of tumor activity. The inter-individual differences in the low tryptophan group, however, were substantial.

Conclusions: In a subgroup of the patients suffering from metastatic carcinoid disease the cerebral access of plasma tryptophan is impaired, thus rendering cerebral serotonin neurotransmission suboptimal and leading to hypercortisolism. The present study provides further support to the idea that low serotonergic function is a risk for developing stress-associated psychopathology.

11. Zoe S.K. Lee, Julian A.J.. et al. Urinary Epinephrine and Norepinephrine Interrelations With Obesity, Insulin, and the Metabolic Syndrome in Hong Kong Chinese. *Metabolism*, Vol 50, No 2 (February), 2001: pp 135-143.

The metabolic syndrome is characterized by a clustering of cardiovascular risk factors including type 2 diabetes mellitus, hypertension, dyslipidemia, and obesity. Elevated plasma insulin and urinary norepinephrine (noradrenaline) and reduced urinary epinephrine (adrenaline) excretion are associated with obesity in Caucasian populations. We examined the interrelationships between obesity, plasma insulin, and urinary catecholamine excretion in Chinese subjects with various components of the metabolic syndrome. A total of 577 Chinese subjects (aged 38 6 10 years; 68% with type 2 diabetes mellitus, hypertension, dyslipidemia, obesity, and/or albuminuria and 32% healthy subjects) were studied, all of whom had a plasma creatinine less than 150 mmol/L. The blood pressure, height, weight, waist and hip circumference, and fasting plasma glucose,

insulin, lipid, and creatinine levels were measured. A 24-hour urine sample was collected for measurement of albumin and catecholamine excretion. The body mass index (BMI) and waist circumference were used as measures of general and central obesity, respectively. The insulin resistance index was estimated by the calculated product of fasting plasma insulin and glucose concentrations. Patients with an increasing number of components of the metabolic syndrome (type 2 diabetes mellitus, hypertension, dyslipidemia, obesity, and/or albuminuria) were more obese, hyperglycemic, dyslipidemic, and albuminuric and had higher blood pressure, plasma insulin, insulin resistance indices, and 24-hour urinary norepinephrine excretion but lower urinary epinephrine output (all P < .005). Increasing quintiles of BMI in the whole population or waist circumference in both sexes were associated with increasing trends for adverse lipid

profiles, plasma insulin, insulin resistance indices, and urinary norepinephrine excretion but a decreasing trend for urinary epinephrine output (all $P < .001$). There were close associations between age, obesity, blood pressure, fasting plasma glucose, lipid, insulin, insulin resistance indices, and urinary catecholamine excretion. Using stepwise multiple regression analysis (all $P < .001$), 34% of the variability of the BMI and 45% of that of the waist circumference were independently related to gender (waist higher in males and BMI higher in females), increased plasma insulin, triglyceride, and urinary norepinephrine excretion, and decreased high-density lipoprotein (HDL) cholesterol and urinary epinephrine output. In Chinese subjects with different manifestations of the metabolic syndrome, hyperinsulinemia, insulin resistance, elevated norepinephrine, and reduced epinephrine excretion were closely associated with general and central obesity. Based on these findings, we postulate that complex interactions between the insulin and sympathoadrenal systems may lead to the development of obesity and the metabolic syndrome.

12. Trachte, G; Uncini T.; Hinz, M. Both stimulatory and inhibitory effects of dietary 5-hydroxytryptophan and tyrosine are found on urinary excretion of serotonin and dopamine in a large human population. *Neuropsychiatric Disease and Treatment*, 2009;5:227-235.

Amino acid precursors of dopamine and serotonin have been administered for decades to treat a variety of clinical conditions including depression, anxiety, insomnia, obesity, and a host of other illnesses. Dietary administration of these amino acids is designed to increase dopamine and serotonin levels within the body, particularly the brain. Convincing evidence exists that these precursors normally elevate dopamine and serotonin levels within critical brain tissues and other organs. However, their effects on urinary excretion of neurotransmitters are described in few studies and the results appear equivocal. The purpose of this study was to define, as precisely as possible, the influence of both 5-hydroxytryptophan (5-HTP) and tyrosine on urinary excretion of serotonin and dopamine in a large human population consuming both 5-HTP and tyrosine. Curiously, only 5-HTP exhibited a marginal stimulatory influence on urinary serotonin excretion when 5-HTP doses were compared to urinary serotonin excretion; however, a robust relationship was observed when alterations in 5-HTP dose were compared to alterations in urinary serotonin excretion in individual patients. The data indicate three statistically discernible components to 5-HTP responses, including inverse, direct, and no relationships between urinary serotonin excretion and 5-HTP doses. The response to tyrosine was more consistent but primarily yielded an unexpected reduction in urinary dopamine excretion. These data indicate that the urinary excretion pattern of neurotransmitters after consumption of their precursors is far more complex than previously appreciated. These data on urinary neurotransmitter excretion might be relevant to understanding the effects of the precursors in other organs.

13. Pussard, E; Neveux, M; Guigueno, N. Reference intervals for urinary catecholamines and metabolites from birth to adulthood. *Clinical Biochemistry*, 42 (2009) pp. 536-539.

Objective: To establish reference intervals for urinary excretion of biogenic amines from birth to adulthood. Design and methods: 865 outpatients were categorized into nine groups ranging from birthday to 25 years. Free catecholamines, total metanephrines, vanillylmandelic and homovanillic acids were determined in urine samples by HPLC with amperometric detection.

Results: The ratio of each analyte-to-creatinine declined gradually from birth to 15–18 years reaching adults values. No difference was observed by sex except a lower excretion of epinephrine and metanephrine in girls than in boys below 1 year.

Conclusion: Our data provide age-appropriate reference ranges for the diagnosis of tumors from neural crest in children.

14. S. Winterkamp, M.D., M. Weidenhiller, M.D., P. Otte, M.D., J. Stolper, M.D., D. Schwab, M.D. Urinary Excretion of N-Methylhistamine as a Marker of Disease Activity in Inflammatory Bowel Disease. *The American Journal of Gastroenterology*, Vol. 97, No. 12, 2002, :3071–3077.

OBJECTIVE: Mast cells are thought to participate in the pathogenesis of inflammatory bowel disease (IBD). In this study, urinary excretion of N-methylhistamine (UMH), a stable metabolite of the mast cell mediator histamine, was evaluated as an indicator of disease activity in patients with IBD.

RESULTS: Urinary excretion of UMH was found to be significantly elevated in IBD. Patients with active Crohn's disease (7.1 ± 4.2 , $p = 0.002$ vs controls) and active ulcerative colitis (8.1 ± 4.8 , $p = 0.02$ vs controls) had higher rates of UMH excretion than patients in remission (6.3 ± 3.8 and 5.2 ± 2.3 , respectively) or controls (4.6 ± 1.9). In Crohn's disease and ulcerative colitis, a significant correlation of UMH excretion with clinical disease activity was obtained (Crohn's Disease Activity Index $r^2 = 0.58$, Clinical Activity Index $r^2 = 0.57$, $p = 0.0001$). Serologically, orosomucoid showed the best positive correlation with disease activity (Crohn's Disease Activity Index $r^2 = 0.80$, Clinical Activity Index $r^2 = 0.86$, $p = 0.0001$), but UMH excretion was found to reflect disease activity more accurately than C-reactive protein (Crohn's Disease Activity Index $r^2 = 0.46$, Clinical Activity Index $r^2 = 0.42$, $p = 0.0001$). No association between UMH excretion and disease type or localization could be found in Crohn's disease. However, UMH excretion correlated strongly with endoscopic severity of inflammation in Crohn's disease (Crohn's Disease Endoscopic Index of Severity $r^2 = 0.70$, $p = 0.0001$) or disease extent in ulcerative colitis.

CONCLUSIONS: Urinary excretion of the histamine metabolite UMH is enhanced in IBD. It appears to represent an integrative parameter to monitor clinical and endoscopic disease activity in IBD, which appears to be influenced most likely by mediators released from histamine-containing cells, such as intestinal mast cell subtypes.

15. Baranowska, I, Plonka, J. Determination of Biogenic Amines and Vitamins in Urine Samples with HPLC. *Journal of Liquid Chromatography & Related Technologies*, 31: 2974-2987, 2008.

An HPLC system for simultaneous separation and marking of biogenic amines and their metabolites from catecholamines group: dopamine (DA), epinephrine (E), normetanephrine (NMN), metanephrine (MN), 3,4-dihydroxyphenylacetic acid (DOMA), 3-methoxy-4-hydroxyphenyl-glycol (MHPG), homovanillic acid (HVA) and indoloamines group: serotonin (5HT) and 5-hydroxyindole-3-acetic acid (5HIAA), as well as water soluble vitamins B-1, B-2, B-3, B-6, B-12, and C has been developed. The developed system has been used for marking the examined compounds in urine samples.

16. Persson, M. Aberrant amino acid transport in fibroblasts from patients with bipolar disorder. *Neuroscience Letters*, Vol: 457, 1, 2009, pp: 49-52

Aberrant tyrosine transport is a repeated finding in fibroblasts from schizophrenic patients. The transport aberration could lead to disturbances in the dopaminergic and noradrenergic neurotransmitter systems. Tyrosine and tryptophan are the precursors of the neurotransmitters dopamine and serotonin. Disturbed dopaminergic, noradrenergic and serotonergic systems are implicated as causes of bipolar disorder. Hence, the aim of this study was to explore whether patients with bipolar disorder have an aberrant transport of tyrosine and/or tryptophan. Fibroblast cell lines from patients with bipolar type-1 disorder (n=10) and healthy controls (n=10) were included in this study. All patients fulfilled the DSM-IV diagnostic criteria. The transport of amino acids across the cell membranes was measured by the cluster tray method. The kinetic parameters, maximal transport velocity (V(max)) and affinity constant (K(m)) were determined. A significantly lower V(max) for tyrosine (p=0.027) was found in patients with bipolar type-1 disorder in comparison to healthy controls. No significant differences in K(m) for tyrosine and in the kinetic parameters of tryptophan between patients with bipolar type-1 disorder and healthy controls were observed.

The decreased tyrosine transport (low V(max)) found in this study may indicate less access of dopamine in the brain, resulting in disturbed dopaminergic and/or noradrenergic neurotransmission, that secondarily could lead to disturbances in other central neurotransmitter systems, such as the serotonergic system. However, as sample size was small in this study and an age difference between patients and controls existed, the present findings should be considered as pilot data. Further studies with larger sample number are needed to elucidate the transport aberration and the significance of these findings.

17. Fernell, E. Karagiannakis, A; Edmanc, G. et al. Aberrant amino acid transport in fibroblasts from children with autism. *Neuroscience Letters*, 418 (2007) 82–86.

Autism is a developmental, cognitive disorder clinically characterized by impaired social interaction, communication and restricted behaviours. The present study was designed to explore whether an abnormality in transport of tyrosine and/or alanine is present in children with autism. Skin biopsies were obtained from 11 children with autism (9 boys and 2 girls) fulfilling the DSM-IV diagnostic criteria for autistic disorder and 11 healthy male control children.

Transport of amino acids tyrosine and alanine across the cell membrane of cultured fibroblasts was studied by the cluster tray method. The maximal transport capacity, Vmax and the affinity constant of the amino acid binding sites, Km, were determined. Significantly increased Vmax for alanine (p = 0.014) and increased Km for tyrosine (p = 0.007) were found in children with autism. The increased transport capacity of alanine across the cell membrane and decreased affinity for transport sites of tyrosine indicates the involvement of two major amino acid transport systems (L- and A-system) in children with autism. This may influence the transport of several other amino acids across the blood–brain-barrier. The significance of the findings has to be further explored.

18. D. Tomasia, R.Z. Goldsteina, F. Telanga, T. Maloneya, N. Alia-Kleina, E.C. Caparellia. Widespread disruption in brain activation patterns to a working memory task during cocaine abstinence. *Brain Research*, 1171 pp 83-92, 2007.

Cocaine abstinence is associated with impaired performance in cognitive functions including attention, vigilance and executive function. Here we test the hypothesis that cognitive dysfunction during cocaine abstinence reflects in part impairment of cortical and subcortical regions modulated by dopamine. We used functional magnetic resonance

imaging (fMRI) to study brain activation to a verbal working memory task in cocaine abusers (n=16) and healthy controls (n=16). Compared to controls, cocaine abusers showed: (1) hypoactivation in the mesencephalon, where dopamine neurons are located, as well as the thalamus, a brain region involved in arousal; (2) larger deactivation in dopamine projection regions (putamen, anterior cingulate, parahippocampal gyrus, and amygdala); and (3) hyperactivation in cortical regions involved with attention (prefrontal and parietal cortices), which probably reflects increased attention and control processes as compensatory mechanisms. Furthermore, the working memory load activation was lower in the prefrontal and parietal cortices in cocaine abusers when compared with controls, which might reflect limited network capacity. These abnormalities were accentuated in the cocaine abusers with positive urines for cocaine at time of study (as compared to cocaine abusers with negative urines) suggesting that the deficits may reflect in part early cocaine abstinence. These findings provide evidence of impaired function of regions involved with executive control, attention and vigilance in cocaine abusers. This widespread neurofunctional disruption is likely to underlie the cognitive deficits during early cocaine abstinence and to reflect involvement of dopamine as well as other neurotransmitters.

19. Marit A.C. Tanke, Ido P. Kema, J. Dijck-Brouwer, Bennard Doornbos, Elisabeth G.E. De Vries, J. Korf. Low plasma tryptophan in carcinoid patients is associated with increased urinary cortisol excretion. *Psychoneuroendocrinology* (2008) 33, 1297—1301.

Background: Previously we observed in patients suffering from a metastatic carcinoid tumor that irritability, aggression and lack of impulse control are associated with low levels of plasma tryptophan and presumably with low brain serotonin function. In rats we showed that a diet of low tryptophan resulted in higher stress responses and higher corticosterone production. Here we tested in carcinoid patients whether tryptophan depletion due to tumor 5-HT overproduction is associated with high cortisol production.

Methods: Urinary excretion of cortisol, serotonin, 5-hydroxyindole acetic acid (the main metabolite of serotonin a marker of tumor activity), plasma levels of tryptophan and platelet content of serotonin (index of peripheral serotonin synthesis) were determined in metastatic midgut carcinoid patients. Patients (N = 25) were divided into two groups based on their plasma tryptophan levels (≤ 25 mmol/l, n = 12 and ≥ 49 mmol/l, n = 13).

Results: Carcinoid patients with low plasma tryptophan levels had significantly higher urinary excretion of free cortisol ($p < 0.01$), independent of tumor activity. The inter-individual differences in the low tryptophan group, however, were substantial.

Conclusions: In a subgroup of the patients suffering from metastatic carcinoid disease the cerebral access of plasma tryptophan is impaired, thus rendering cerebral serotonin neurotransmission suboptimal and leading to hypercortisolism. The present study provides further support to the idea that low serotonergic function is a risk for developing stress-associated psychopathology.

20. Rouveix, M.; Duclos, M.; Gouarne, C.; Beauvieux, M.C. The 24 h Urinary Cortisol/Cortisone Ratio and Epinephrine/Norepinephrine Ratio for Monitoring Training in Young Female Tennis Players. *Int J Sports Med*, 2005, 27: 856-863.

The effect of training variations on the 24 h urinary cortisol/cortisone (C/Cn) ratio and the epinephrine/norepinephrine (E/NE)

ratio in relation with mood (evaluated using the Brunel Mood Scale: BRUMS) and performance was investigated in seven trained young female tennis players (12.8 ± 1.7 years). Like the proposed model in adults, the monitoring of hormonal and mood parameters could be a useful index in training follow-up in young sportswomen. Assessment of nutritional intake, nitrogen excretion rate and nitrogen balance were also determined to measure the dietary practice of these athletes. Nitrogen balance was calculated from the mean daily protein intake and the urinary nitrogen excretion. Data were collected after a 1-month rest (September, T1), 3 months after T1 (after technical and endurance training: December, T2) and 7 months after T1 (after 4 months of increasing-volume/high-intensity training: March, T3). A significant increase in C/Cn ratio (+ 30%, $p < 0.05$) were noted from T1 to T3. In the same time, urinary NE concentrations decreased significantly. The E/NE ratio increased from T1 to T2 and decreased at T3 (T1 vs. T3: - 30%, $p < 0.05$). The BRUMS inventory at T3 reflected changes in specific mood states with a significant increase in fatigue and anger scores, while vigor scores decreased significantly compared to T1. This period also corresponded with the lowest percentage of matches won and with the highest training load. Energy intake was about 16% lower than the French recommendations for girls of the same age. However, a positive nitrogen balance was observed from a mean intake of $1.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$. Our results reveal that an increase of overnight urinary C/Cn ratio and a decrease of E/NE ratio are concomitant with alterations in mood state and performance, all these parameters being associated with physical and psychological stress.

21. Ayelet B. Snow, A. Khalyfa, Laura D. Serpero, O. Capdevila, Jinkwan Kim, M.O. Buazza, and David Gozal. Catecholamine Alterations in Pediatric Obstructive Sleep Apnea: Effect of Obesity. *Pediatric Pulmonology*, 44: 559-567, 2009.

Summary. Study Objectives: Obstructive sleep apnea (OSA) elicits increased sympathetic activity in adults and increased urinary catecholamines. Moreover, urinary catecholamine excretion is altered in obese patients. We hypothesized that morning urine catecholamine levels would be correlated with the severity of obstructive sleep apnea and degree of obesity in children.

Methods: Children referred to the pediatric sleep center for habitual snoring underwent overnight polysomnography, and the first morning voided urine sample was collected. Urinary concentrations of norepinephrine, epinephrine and dopamine were measured and corrected for creatinine levels. In a subset of children, blood samples were drawn and gene expression of catecholamine-relevant genes analyzed by quantitative real-time PCR. Results: One hundred fifty-nine children were recruited and completed the protocol. Children with OSA had significantly higher urinary norepinephrine and epinephrine levels, but not dopamine, compared to habitual snorers (norepinephrine: $40.1_{-24.7}$ ng/mg creatinine vs. $31.6_{-16.2}$ ng/mg creatinine, $P < 0.01$; epinephrine: $6.4_{-10.5}$ ng/mg vs. $4.5_{-0.5}$ ng/mg, $P < 0.01$). There was a positive

correlation between norepinephrine and epinephrine values and polysomnographic indices, but no effect of obesity on catecholamine levels. In addition, expression of several of the major genes involved in synthesis and transport of catecholamines, as well as in selected receptors were compatible with increased bioavailability of catecholamines.

Conclusions: In children with OSA, morning urinary norepinephrine and epinephrine levels are significantly higher than those without OSA, and correlate with the severity of the disease. Gene expression patterns are in agreement with such findings. Urine catecholamine levels do not appear to be influenced by the presence of obesity. Thus, altered sympathetic activity in OSA patients appears to occur independently of the presence of obesity.

22. G. De Pergola¹, F. Giorgino, Ritanna Benigno¹, Pietro Guida¹ and Riccardo Giorgino. Independent Influence of Insulin, Catecholamines, and Thyroid Hormones on Metabolic Syndrome. *Obesity* (2008) 16, 2405–2411.

The objective of this study was to examine whether metabolic syndrome, defined according to adult treatment panel III criteria, is associated with insulin, catecholamines, and thyroid hormones, independently of age and gender. A cohort of 651 euthyroid overweight and obese patients, 440 women and 211 men, aged 18–68 years, were examined. Central fat accumulation (indirectly measured by waist circumference), fasting thyroid-stimulating hormone (TSH), FT3, FT4, insulin, glucose, and lipid (cholesterol, HDL-cholesterol, and triglyceride) serum concentrations, 24-h urinary catecholamines, and the level of insulin resistance (estimated by homeostasis model assessment for insulin resistance (HOMAIR)) were measured. Patients with metabolic syndrome showed higher insulin ($P < 0.001$) and FT3 ($P < 0.001$) serum levels and higher 24-h urinary noradrenaline ($P < 0.001$) than subjects without this syndrome. The number of metabolic syndrome parameters was directly associated with insulin ($P < 0.001$) and FT3 ($P < 0.05$) serum levels, and with 24-h urinary noradrenaline ($P < 0.001$) in the whole population.

When a multiple regression analysis was performed with the metabolic syndrome as the dependent variable, and age, gender, and insulin, and TSH, FT3, FT4 serum levels, and 24-h urinary noradrenaline and adrenaline as independent variables, the metabolic syndrome maintained an independent positive association with age ($P < 0.001$), male sex ($P < 0.001$), insulin ($P < 0.001$), and 24-h urinary noradrenaline ($P < 0.001$). In conclusion, this study suggests that insulin and noradrenaline cooperate independently to the development of the metabolic syndrome.

23. A. Elmasry^{*,#}, E. Lindberg^{*}, J. Hedner[†], C. Janson^{*}, G. Boman. Obstructive sleep apnoea and urine catecholamines in hypertensive males: a population-based study. *Eur Respir J*, 2002; 19: 511–517.

ABSTRACT: Studies addressing the relationship between obstructive sleep apnoea (OSA) and sympathoadrenal activity have been criticized for poor control of factors known to confound sympathetic function, including hypertension. The aim of this study was to investigate the relationship between OSA and urinary catecholamines in a population-based sample of hypertensive males. In 1994, 2,668 males aged 40–79 yrs answered a questionnaire regarding sleep disorders and somatic diseases. Of those who reported hypertension, an age-stratified sample of 116 was selected for monitoring of breathing during sleep and overnight urine analysis.

Subjects with OSA, defined as apnoea-hypopnoea index ≥ 10 h⁻¹, had higher concentrations of urinary normetanephrine (182;57 versus 141;45 mmol·mol⁻¹ creatinine, *p*<0.001) and metanephrine (70;28 versus 61;28 mmol·mol⁻¹ creatinine, *p*<0.05) in comparison to subjects without OSA. In a multiple regression analysis, there was an association between variables of sleep-disordered breathing and normetanephrine and metanephrine concentrations, independent of major confounding factors. The authors concluded that, in a population-based sample of hypertensive males, obstructive sleep apnoea is associated with increased urinary concentrations of extraneuronal metabolites of catecholamines independent of major confounding factors, suggesting increased sympathoadrenal activity. Elevated sympathoadrenal activity may explain the increased cardiovascular morbidity associated with obstructive sleep apnoea.

24. Whiting, M J. Simultaneous measurement of urinary metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection. *Ann Clin Biochem.* Volume: 46, Issue: Pt 2, 2009 pp: 129-36.

Summary: BACKGROUND: The measurement of catecholamines and metanephrines in [urine](#) is an important diagnostic test in biochemical screening for pheochromocytoma. Tandem mass spectrometry (MSMS) has the potential to be used in a profiling method for simultaneous assay of these analytes. METHODS: Optimal conditions were established for the MSMS detection of catecholamines (noradrenalin, adrenalin and dopamine) and metanephrines (normetanephrine and metanephrine), including commercially available isotopically labelled compounds for use as internal standards. Chromatographic separation of all five polar biogenic amines was achieved under solvent conditions that were compatible with MSMS and multiple reaction monitoring. Several types of solid-phase extraction cartridge were used to investigate clean-up conditions for [urine](#), and acid-hydrolysates of [urine](#), prior to LC-MSMS. RESULTS: Total catecholamines and metanephrines from acid-hydrolysed urines, or free catecholamines and free metanephrines from native urines, were complexed with diphenyl-boronate and recovered in high yield from polymer cartridges after elution with formic acid. Direct injection of eluates into the LC-MSMS system allowed quantitation of catecholamines and metanephrines with a run time of 6 min per sample. Biogenic amine concentrations for patient urines and quality assurance programme samples, and assay imprecision, were similar to values obtained with high-performance liquid chromatography methods, which used electrochemical detection. In normal urines, the ratio of free to total catecholamines was around three-fold higher than the ratio of free to total metanephrines. CONCLUSION: The assay of urinary catecholamines and metanephrines can be achieved simultaneously using one LC-MSMS method, which is rapid and reduces labour and consumable costs for routine application. Subject: Catecholamines [urine](#)

25. Sofuoglu, Mehmet. Norepinephrine and stimulant addiction. *Addiction Biology*, Vol. 14, no. 1, pp. 119-129. Jan 2009.

Summary: No pharmacotherapies are approved for stimulant use disorders, which are an important public health problem. Stimulants increase synaptic levels of the monoamines [dopamine](#) (DA), serotonin and norepinephrine (NE). Stimulant reward is attributable mostly to increased DA in the reward circuitry, although DA stimulation alone cannot explain the rewarding effects of stimulants. The noradrenergic system, which uses NE as the main chemical

messenger, serves multiple brain functions including arousal, attention, mood, learning, memory and stress response. In pre-clinical models of addiction, NE is critically involved in mediating stimulant effects including sensitization, drug discrimination and reinstatement of drug seeking. In clinical studies, adrenergic blockers have shown promise as treatments for cocaine abuse and dependence, especially in patients experiencing severe withdrawal symptoms. Disulfiram, which blocks NE synthesis, increased the number of cocaine-negative urines in five randomized clinical trials. Lofexidine, an alpha 2-adrenergic agonist, reduces the craving induced by stress and drug cues in drug users. In addition, the NE transporter (NET) inhibitor atomoxetine attenuates some of d-amphetamine's subjective and physiological effects in humans. These findings warrant further studies evaluating noradrenergic medications as treatments for stimulant addiction.

26. Duhon, A. The role of excitatory and inhibitory neurotransmitters in fibromyalgia. *Dissertation Abstracts Inter. Vol. 68, no. 01, suppl. B, p. 101, 2007.*

Summary: While the etiological roots of fibromyalgia syndrome remain a mystery, numerous studies have pointed to an imbalance in the **neurotransmitter** system. An imbalance between excitatory and inhibitory neurotransmission can manifest itself in both physiological and psychological symptomology. This study examined the role of **neurotransmitter** imbalances and suggests a possible neurochemical relationship between anxiety, fibromyalgia and to a lesser extent depression. **Urinalysis** was used to measure **neurotransmitter** levels from 10 women between the ages of 25-75, all with a clinical diagnosis of fibromyalgia. Anxiety, depression, and fibromyalgia status were assessed using the Beck Anxiety Inventory, Beck Depression Inventory-II, and the Fibromyalgia Impact Questionnaire respectively. Descriptive statistics and Pearson r were computed to examine the data. Findings indicated a pattern of **neurotransmitter** imbalances among subjects. **One hundred percent of subjects displayed deficiencies in both serotonin and epinephrine.** There was also a positive relationship between FIQ scores and BAI scores, and between BAI scores and **neurotransmitter** levels. No relationship was found between FIQ scores and BDI scores, or between FIQ scores and **neurotransmitter** levels. Clinical implications and suggestions for future research are discussed.