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## Serotonin, cortisol, and stress-related psychopathology

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## **Chapter 6**

### **Low plasma tryptophan in carcinoid patients is associated with increased urinary cortisol excretion**

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## **ABSTRACT**

**Objective:** *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 blood plasma tryptophan and presumably with low brain serotonin (5-HT) 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-HT), 5-hydroxyindoleacetic acid (5-HIAA, main metabolite of 5-HT, marker of tumor activity), plasma levels of tryptophan and platelet content of serotonin (index of peripheral 5-HT synthesis) were determined in metastatic midgut carcinoid patients. Patients (N = 25) were divided into two groups based on their plasma tryptophan levels ( $\leq 25 \mu\text{mol/l}$ ,  $n=12$  and  $\geq 49 \mu\text{mol/l}$ ,  $n=13$ ).*

**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.*

**Conclusion:** *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.*

## INTRODUCTION

Lasting tryptophan depletion is found in patients suffering from a metastatic serotonin producing carcinoid tumor (271-274). Carcinoid tumors are rare, malignant but slowly growing tumors that arise from neuro-endocrine cells in the body. Carcinoid tumors secrete a variety of biochemical factors, including serotonin (5-hydroxytryptamine, 5-HT, and 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5-HT. By producing 5-HT, the carcinoid uses up to 60% of the body supplies of its precursor tryptophan (134). This can result in decreased peripheral tryptophan levels and decreased cerebral availability of tryptophan. Because 5-HT cannot cross the blood brain barrier, consequently, the cerebral synthesis and neuronal release of 5-HT also become limited (48).

Chronic depletion of tryptophan as observed in somatic diseases coincides with an increased risk of depression, irritability and aggression (115;116;140). These symptoms are also associated with alteration in the Hypothalamus-Pituitary-Adrenal (HPA)-axis (28;42;87;275;276). For example, increased levels of cortisol in plasma, saliva and urine are found in approximately 50% of the patients suffering from a major depressive disorder (28;30;31). When patients recover from the depressive episode, these abnormalities in the HPA-axis activity disappear. Moreover, patients who still have elevated cortisol levels after recovery have an increase risk of relapse (39).

The relationship between the serotonergic system and the HPA-axis, however, is complex. Not only does serotonergic activity influence HPA-axis function (277), but stress and increased HPA-axis activity can - in turn - affect 5-HT function (88). Furthermore, the effects of chronic and acute alterations in 5-HT are not necessarily the same. Drugs that increase 5-HT function initially stimulate the release of Adrenocorticotrophic Hormone (ACTH) and corticosteroids (55;77-80), while long-term use of antidepressive medication suppresses HPA-axis activity (41;93;94;278).

In the present study, we investigate the effect of long-lasting depletion of tryptophan in carcinoid patients on HPA-axis activity. Urinary 5-HIAA concentration is taken as a marker of tumor activity. Platelet content of 5-HT also served as an index of the peripheral synthesis of 5-HT. To our knowledge, there are no clinical studies that link chronic tryptophan depletion with the corticosteroid state in humans.

## MATERIALS & METHODS

We retrospectively identified 13/12 patients with normal/low plasma tryptophan levels respectively in patients with a clinical and biochemically established metastatic carcinoid tumor in the period 2002-2004 at the Department of Medical Oncology, University Medical Center Groningen, The Netherlands. Patients were divided into two subgroups according to their plasma tryptophan levels. The low tryptophan group was defined as having plasma

tryptophan levels of 25  $\mu\text{mol/l}$  or less, defined as  $>2$  SD below the group mean according to Kema et. al (166). The normal tryptophan group had plasma tryptophan levels of 49  $\mu\text{mol/l}$  or more. There was no difference in age ( $61 \pm 10$  years vs.  $64 \pm 6$  years), male/female ratio (4/9 vs. 6/6), nor in chronicity of the disease ( $7 \pm 1$  years vs.  $7 \pm 2$  years) between both groups. The standard treatment of these patients with serotonin overproduction consists of octreotide, which was used in 8 patient in the normal tryptophan group and 9 patients in the low tryptophan group. Five patients, all in the low tryptophan group were treated with interferon- $\alpha$ . Antidepressants were given to one patient (of the low tryptophan group; mirtazepine).

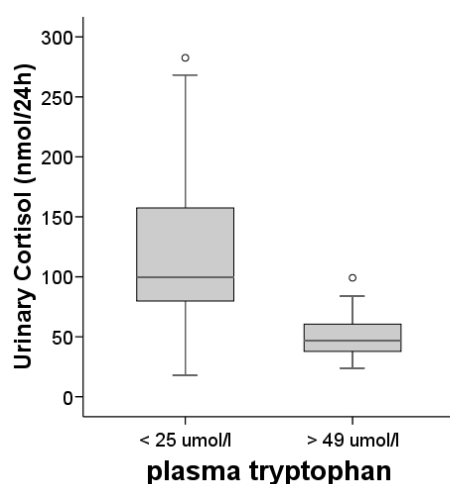
Blood and 24h urine samples were obtained once during routine lab investigations during regular morning visits to the outpatient clinic, as described before (279). Plasma Trp and platelet 5-HT concentrations were measured by means of High Performance Liquid Chromatography (HPLC) with fluorometric detection (166). Reference values for plasma Trp are 40-70  $\mu\text{mol/l}$  (166). The cut off value for platelet 5-HT is 5.4  $\text{nmol}/10^9$  platelets (279). Urinary serotonin and 5-HIAA concentrations were determined with HPLC / fluorometric detection as described previously (279;280). 5-HIAA levels in 24h urine were taken as measure for tumor activity (281). 5-HIAA is the main metabolite and end-product of serotonin. Cortisol excretion was determined in a urine sample over 24 hours. Urine was purified and free cortisol was measured with a validated routinely used ElectroChemiLuminescence ImmunoAssay (ELECSYS, Roche Diagnostics Corporation, Hague Rd Indianapolis), with streptavidin microparticles. Cortisol was measured in urine to obtain a measure of total cortisol excretion per day. This approach also circumvents variations due to sampling time. Patients were experienced in collecting 24 h urine as this is done routinely twice a year when they visit the Department of Medical Oncology in the UMCG. Reference values for cortisol excretion are 20-270  $\text{nmol}/24$  h.

Statistical analyses were done with SPSS (version 12.0), with  $p \leq 0.05$  as significant. Plasma concentrations of tryptophan and 5-HT, urinary contents of cortisol, 5-HT and 5-HIAA were analyzed using a Mann-Whitney U test. Correlations were noted using Spearman's correlation coefficient. Results are presented as mean  $\pm$  S.E.M.

## RESULTS

Subjects with low plasma tryptophan had significantly higher urinary 24h cortisol excretion (in  $\text{nmol}/24\text{h}$ ) compared to subjects with normal plasma tryptophan levels ( $Z -2.825$ ;  $p = 0.004$ , figure 1). In the low tryptophan group 3 patients excreted 5 times more cortisol than the other subjects. When these patients are excluded the increase in cortisol excretion was still significantly higher in the low-tryptophan group (Normal Trp (N = 13):  $51 \pm 6$  vs. Low Trp (N = 9):  $82 \pm 1$ ;  $Z - 2.170$   $P = 0.03$ ). The patients that were treated with interferon did not have different cortisol excretion than the other patients in the low tryptophan group. There were no significant differences between the normal and low tryptophan group in platelet 5-

HT ( $17.3 \pm 12.9$  vs.  $19.2 \pm 12.6$  nmol/ $10^9$ platelets), urinary 5-HT ( $1.28 \pm 1.75$  vs.  $17.2 \pm 34.9$   $\mu$ mol/24h) and urinary 5-HIAA ( $0.29 \pm 0.57$  vs.  $0.76 \pm 0.73$  mmol/24h) levels.



**Figure 1:** 24-hours urinary excretion of cortisol in carcinoid patients with low or normal plasma tryptophan levels. (Z -2.825, p = 0.008)

Correlations between biochemical parameters are shown in table 1. Plasma tryptophan levels were negatively correlated with urinary cortisol excretion. There was a correlation between cortisol excretion and urinary 5-HT content but cortisol excretion was not correlated with urinary 5-HIAA levels. In contrast with prior observations, we found a positive correlation between plasma tryptophan and urinary 5-HIAA (166). However, because this correlation was weak and changed into a negative correlation when excluding the three cases with the highest urinary cortisol levels (see above), we believe that this is not a relevant finding, but merely a statistical coincidence.

**Table 1** Correlations between biochemical parameters

	<b>Plasma Tryptophan</b>	<b>Platelet 5-HT</b>	<b>Urinary 5-HIAA</b>	<b>Urinary 5-HT</b>	<b>Urinary Cortisol</b>
<b>Plasma Tryptophan</b> ( $\mu$ mol/L)	1	-0.307	0.483*	-0.502*	-0.609**
<b>5-HT</b> (nmol/ $10^9$ platelet)	-0.307	1	0.773**	0.693**	0.108
<b>5-HIAA Urine</b> (mmol/24h)	0.483*	0.773**	1	0.879**	0.337
<b>5-HT Urine</b> ( $\mu$ mol/24h)	-0.502*	0.693**	0.879**	1	0.522**
<b>Cortisol Urine</b> (nmol/24h)	-0.609**	0.108	0.337	0.522**	1

The positive but weak correlation between plasma tryptophan and urinary 5-HIAA changed into a negative correlation when excluding three cases with the highest urinary cortisol levels, therefore we believe that this is not a relevant finding, but merely a statistical coincidence. Spearman's rho \*p<0.05, \*\*p<0.01

## DISCUSSION

This study confirms our hypothesis that chronic tryptophan depletion in patients suffering from metastatic carcinoid disease is associated with increased cortisol secretion. The data also indicate that the variation in cortisol excretion is far greater in the low tryptophan group as compared to subjects with normal levels of plasma tryptophan.

The strength of this study is that we are able to demonstrate an association between chronic tryptophan depletion and HPA-axis activity in humans. However, carcinoid tumors are rare and the cross-sectional retrospective design of our study in a small patient groups has some drawbacks. First, we do not have longitudinal tryptophan levels. It can be speculated that the patients with the highest urine cortisol levels are the patients with the highest chronic tryptophan depletion or the steepest decline. Second, metastatic carcinoid disease is a stressor that itself may lead to increased production of cortisol. It appeared, however, that tumor activity, as measured by urinary 5-HIAA levels, did not correlate with cortisol levels, suggesting that tumor activity itself did not dictate high cortisol excretion in the low plasma tryptophan group. Third, we cannot exclude that tryptophan depletion is caused by underlying tumor-related causes that itself may alter cortisol excretion, such as reductions in food intake or alterations in immune activation and accordingly indoleamine 2,3-dioxygenase (IDO) activation (282). Decreased dietary intake may decrease plasma levels of tryptophan (47), but not in the extent that is seen in carcinoid patients (283). The carcinoid tumor has an extremely high serotonin production and content which drains significantly on the tryptophan pool (134;284). Fourth, cortisol excretion could be affected by medication. In the low tryptophan group, there was no difference in cortisol excretion between patients with and patients without interferon- $\alpha$  treatment, a cytokine that induces IDO activity and thus lowers tryptophan levels. But because these groups are small, the possibility that the difference in cortisol excretion is caused by a direct effect of either interferon- $\alpha$  or other types of medication cannot be completely ruled out.

Our study is the first to report increased urinary cortisol excretion being associated with chronic deficiencies in plasma tryptophan levels in humans. Many clinical studies that have focused on the effect of 1 – 12 hours tryptophan depletion reported inconsistent effects on HPA-activity and plasma cortisol (275;285-288). Whereas some (285;286) found increases in CRH in cerebrospinal fluid and plasma cortisol levels, others (275;287) reported decreases in cortisol levels after acute tryptophan depletion. In animal studies, however, the effect of chronic tryptophan manipulation has been studied.(289), here chronic dietary depletion of tryptophan in rats increased resting plasma levels of corticosterone. In addition, dietary supplementation of tryptophan decreases stress evoked cortisol levels in pigs (290). In a animal experiment, decreases in plasma tryptophan only increased plasma corticosterone and adrenal weight in animals which concomitantly received a mild stressor (182). Apparently low plasma tryptophan levels per se do not increase cortisol secretion, but may potentiate the response of the organism to stress, which in turn may activate HPA-activity.

Translating this to the present study, it may well be that suffering from metastatic carcinoid disease is a stressful life event that may lead to increased production of cortisol, particularly in patients with low cerebral availability of tryptophan.

**(Clinical) implications**

We show that chronic depletion of plasma tryptophan levels in carcinoid patients is associated with increased cortisol excretion, and accordingly, increased sensitivity to stress. This might increase the patients' risk to develop stress-related diseases, for example certain psychiatric diseases. Future research should be directed to study the susceptibility to stress and HPA-axis response in humans with chronic low tryptophan levels.

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Chapter 6