# Manipulating serotonin function in depression

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# 6

# Plasma tryptophan levels following low-dose and high-dose tryptophan depletion

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ATD has been found to markedly decrease plasma tryptophan levels and the ratio tryptophan/LNAA, depending on the amount and composition of the amino acid mixture, whether it is combined with a low-tryptophan diet and on the time at which plasma samples are taken. In this chapter, different 'low-dose' ATD methods are discussed. Also, the inter-individual variations in plasma tryptophan levels following high-dose and low-dose ATD are reported.

**Low-dose tryptophan depletion**

Acute tryptophan depletion (ATD) is a popular method to investigate the effects of lowered serotonin function in humans. Acute tryptophan depletion induces a temporary depressive 'relapse' in 50-60% of remitted depressed patients treated with serotonergic antidepressants. In healthy individuals, ATD has no or minor mood effects, but cognitive effects have been found in both healthy and recovered depressed individuals (Booij et al., 2003).

The magnitude of the reduction of plasma tryptophan concentrations following ATD depends on the amount and composition of the amino acid mixture (Young et al., 1989) and whether or not a pre-test low tryptophan diet is included. It has been suggested that a threshold exists that needs to be exceeded before any behavioural effects occur, since studies in which the plasma tryptophan reduction was lower than 70% generally do not find any symptomatic effects (Van der Does, 2001b). However, depression-congruent effects on sleep architecture have been observed at moderate tryptophan reductions (Bhatti et al., 1998). The placebo procedure developed by Krahn et al. (1996) may be suitable as a low-dose ATD procedure (Van der Does, 2001a). Since this procedure reduces plasma tryptophan concentrations by 40-50%, and has been found not to affect mood (Booij et al., 2005a), it allows for the investigation of possible dose-response effects.

Booij et al. (2005a), using the Krahn et al. method as low-dose ATD, found that ATD had a dose-dependent effect on selective attention (Stroop colour-word interference) in remitted depressed patients, but no other cognitive effects of low-dose ATD were observed. Merens et al. (in press) observed no effects of low-dose ATD on attention, memory, and accuracy of emotion recognition in remitted depressed patients (Merens et al., in press). Two recent papers have reported much stronger effects of low-dose ATD. Hayward et al. (2005) found that low-dose ATD had no effects on mood ratings in unmedicated recovered depressed subjects, but that it increased the emotion-potentiated startle reflex, impaired recognition of happy faces and initial recall memory and increased emotional Stroop interference. Some cognitive effects were also observed in healthy controls. Munafò et al. (2006) reported that low-dose ATD slightly increased self-rated depressive symptoms in medicated recovered depressed patients and also increased Stroop interference for social threat words.

The low-dose mixture used by Hayward et al. (2005) and Munafò et al. (2006) consisted of eight amino-acids (31.2g), whereas the Krahn et al. (1996) procedure consists of 15 amino-acids (25.7g). We calculated the plasma tryptophan reductions obtained by Hayward et al. (2005), and found that low-dose ATD decreased plasma tryptophan levels by 73.9% in recovered depressed patients. The tryptophan/large neutral amino acids (LNAA) ratio decreased by 86.9%. This suggests that Hayward et al. (2005) studied *high-dose* ATD rather than low-dose. The reductions cannot be calculated from the report by Munafò et al. (2006), but this study used the same procedure and partly the same sample. Viewing these studies as high-dose ATD studies resolves the inconsistencies with the studies by Booij et al. (2005a) and Merens et al. (in press). It also explains the symptomatic effects in the Munafò et al.

study. However, high-dose ATD would be expected to have increased symptoms in Hayward et al.'s paper. This may be explained by the fact that patients in this study had a relatively low number of previous episodes, which predicts a weaker response to ATD (Booij et al., 2002).

There is no generally accepted definition of high-dose or low-dose ATD. Hayward et al. (2005) presented their study as a low-dose ATD study on the basis of the amount of amino acids used. However, peripheral biochemical measures indicate that this study may be considered a high-dose ATD study. In our view, the term low-dose should reflect the decrease of plasma tryptophan concentrations and not the amount and content of the ATD mixture. Future research should carefully consider which terminology is used to prevent misinterpretation and biochemical data should be reported in detail.

**Inter-individual variations in plasma tryptophan levels following ATD**

The regularly used ATD mixture containing fifteen amino acids, weighing approximately 100g (also called 'high-dose' ATD) usually results in a temporary decrease in plasma tryptophan levels of 70-90%. Peak reductions in plasma levels are found 5 to 7 hours after ingestion of the amino-acid mixture (Delgado et al., 1990; Young et al., 1985). This decrease in plasma tryptophan levels following ATD is thought to be sufficient to significantly decrease brain serotonin synthesis (Biggio et al., 1974; Young et al., 1989). Since tryptophan competes with the other large neutral amino acids (LNAA) at the blood-brain barrier, the ratio of tryptophan/LNAA is suggested to be an important index of serotonin turnover (Biggio et al., 1974; Fernstrom & Wurtman, 1972).

In our ATD study, which is reported in Chapter 5 and 7, high-dose ATD led to a 91% reduction of plasma tryptophan/LNAA ratio, compared to a 59% reduction following low-dose ATD (: a mixture of fifteen amino acids at

a quarter strength of the regular 100g mixture). Since a sizable number of patients had vomited or not finished the AA mixture, we inspected the individual decreases in plasma tryptophan and the tryptophan/LNAA ratio following both low-dose and high-dose ATD (see Table 1). Especially the low-dose mixture resulted in a large variability in the reductions in the tryptophan/LNAA ratio: three patients showed a large decrease (79% - 86%) in the plasma tryptophan/LNAA ratio, equivalent to the decreases found in the high-dose condition. Three other patients showed a relatively small decrease in the tryptophan/LNAA ratio following low-dose ATD (33% - 37%). These six patients all deviated from the mean decrease following low-dose (59% ± 16) with more than one standard deviation.

Following high-dose ATD, three patients showed a smaller decrease than expected in plasma tryptophan/LNAA ratio (71% - 81%). For two of these patients, this may be attributed to the fact that they vomited following the high-dose mixture. However, three other patients that vomited following the high-dose mixture did not show a smaller than expected decrease in plasma levels.

The inter-individual variations in the decreases of plasma tryptophan/LNAA ratio were larger than in previous studies (Booij et al., 2005a; Booij et al., 2006b). Unfortunately, studies usually do not report individual decreases in plasma tryptophan levels, so not much is known about their variations or the origin of these variations. One factor that could play a role in inter-individual differences in tryptophan levels following ATD is gender. Men have a higher rate of serotonin synthesis compared to women and the biochemical effects of ATD are greater in women compared to men (Nishizawa et al., 1997). The two men that were included in our study did show relatively small decreases in tryptophan/LNAA ratio following low-dose

ATD, but the biochemical response to high-dose ATD in men was similar to women's.

The large variations in tryptophan levels may be partly explained by the fact that several participants vomited. Five out of eighteen patients that were included in our study, vomited in response to high-dose ATD, one of whom also vomited in response to low-dose ATD. Four of these patients were able to complete the study. One patient dropped out after the high-dose ATD session (which was the second session for her) because of an emotional reaction to the depletion drink. One patient was excluded from the analyses because she dropped out after the first session (high-dose ATD) due to physical and mood complaints following that session (she also vomited in response to the high-dose mixture).

Since the present study was a continuation of previous experiments, using the same batch of amino acids, and the same design, procedures, recruitment and setting, it is not certain what has caused the differences in tolerability. However, ATD mixtures are consistently reported to be highly unpalatable and nauseating (Reilly et al., 1997). There are rather large differences in the reported tolerability of ATD in the literature. Some studies do not report any somatic side-effects, other studies may report side effects following both the tryptophan depletion drink and the control mixture (e.g. Rubinsztein et al., 2001; Schmitt et al., 2000; Spillman et al., 2001); other studies report low, but varying numbers of patients that vomited following the ATD mixture (e.g. Anderson et al., 2003; Booij et al., 2005a; Riedel et al., 1999; Spillman et al., 2001).

Despite a clear overall difference between low-dose and high-dose ATD in the present study, in one third of the patients the biochemical effects

were out-of-range following low-dose, and in three out of eighteen patients following high-dose.

**Table 1.** Individual decreases in tryptophan/LNAA ratio following low-dose and high-dose ATD (%)

| Patient | low-dose | high-dose |
|---|---|---|
| 1 | -37.41 | -88.19 |
| 2 | -32.73 | -93.57 |
| 3 | -50.81 | -96.14 |
| 4 | -72.75 | -97.72**a** |
| 5 | -59.36 | -94.63 |
| 6 | -67.11 | -97.59**b** |
| 7 | -71.93 | -98.65 |
| 8 | -60.98 | -96.11 |
| 9 | -85.98 | -98.81 |
| 10 | -49.35 | -80.79 |
| 11 | -50.84 | -97.25 |
| 12 | -48.89 | -96.19 |
| 13 | -79.94 | -96.91 |
| 14 | -79.22 | -87.42**c** |
| 15 | -33.83 | -96.12 |
| 16 | -47.87 | -86.43 |
| 17 | -67.52**d** | -70.55**e** |
| 18 | -55.72 | -72.91**f** |

**a**: vomited five hours after ingestion of the high-dose mixture
**b**: vomited within 15 minutes after ingestion of the high-dose mixture
**c**: vomited one hour after ingestion of the high-dose mixture
**d**: vomited one and a half hour after ingestion of the low-dose mixture
**e**: vomited ten minutes after ingestion of the high-dose mixture
**f**: vomited one and a half hour after ingestion of the high-dose mixture

We underlined the values that differed from the mean decrease in plasma tryptophan/LNAA by more than one standard deviation: low-dose: M = 58.5% SD = 16.0; high-dose: M = 91.4% SD = 8.7.