Of Green Tea, Black Pepper, and Amyloidoses

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After being diagnosed with AL amyloidosis in 2004 and undergoing conventional chemotherapy, Werner Hunstein (WH) began drinking green tea in September, 2006. He had been informed about the results of an investigation that was described in detail later in the journal *Nature* (Ehrnhoefer et al. 2008). This study demonstrated that the main component of green tea, epigallocatechin gallate (EGCG), is able to bind to denatured protein and thereby to inhibit the formation of insoluble amyloid. The proteins investigated in this case were α-synuclein and the amyloid β-protein, which play a role in the development of Parkinson's and Alzheimer's disease.

After half a year of consuming about two litres of green tea daily (10-12 g of green Darjeeling per litre brewed for over 5 minutes at 70 °C), his thickened tongue and the markedly thickened walls of his heart had nearly returned to the norm. He wrote of these positive observations in a letter to the editor of the journal *Blood* (Hunstein 2007).

In an investigation in Hamburg, it was shown that EGCG inhibits the formation of amyloid from a fragment of the prostate acid phosphatase of human semen and that pre-existing amyloid is degraded (Hauber et al. 2009). A laboratory study in Portugal reported that EGCG inhibits the formation of transthyretin amyloid and degrades pre-existing amyloid (Ferreira et al. 2009).

According to a study of commercially available green tea by Henning et al. (2003), 10 g of tea brewed for 3 minutes in one litre of boiling water releases 340 mg EGCG and 260 mg caffeine. A similar result can be assumed for brewing tea at 70 °C for 5 minutes. On this basis, Hunstein ingested 700-800 mg EGCG daily by drinking 2 litres of green tea.

Green tea has been known for over 4000 years. In Southeast Asia a daily consumption of more than 1.5 litres is not unusual. The concept of the "Asian Paradox" refers to the phenomenon that the Asian population has a low mortality due to cardiovascular disease and lung cancer in spite of an extremely high degree of cigarette use (see Sumpio et al. 2006). A high consumption of green tea is thought to be responsible. A prospective study from Japan revealed that a high consumption of green tea is associated with lower overall and cardiovascular-dependent mortality, although the mortality rate due to cancer is the same for high and low tea consumption (Kuriyama et al. 2006). Another (prospective) Japanese study concluded that the risk of death from leukaemia is markedly lowered by consuming of > 0.5 litres of green tea daily. Numerous other effects have been ascribed to green tea and its components. We will not describe these studies in detail here; instead, the reader is referred to the literature.

Harmful effects of a high consumption of green tea are not known or are attributed to the caffeine contained therein. Patients with cardiovascular disease occasionally do not tolerate the caffeine and greater fluid intake associated with high green tea consumption. It has to be taken into account that the so-called systemic amyloidoses lead to a lower absorption of nutrients in the digestive tract, which would also pertain

to EGCG from green tea. Therefore, it would be interesting to determine how a higher plasma level of EGCG could be obtained for the patients mentioned above using less extreme measures.

To this end, green tea extract (GTE) is commercially available in capsule form. Lee et al. (2002) found after administration of 70 mg GTE a maximum EGCG plasma level of 0.05 µmol/L. Henning et al. (2004) determined, following a dose of 190 mg or 0.4 mmol EGCG from GTE, a plasma level of 0.15 µmol/L. Ullmann et al. (2003) found 1.5 hours after administration of 50 to 1600 mg (0.1 to 3.5 mmol) pure EGCG under fasting conditions an average plasma level of 0.3 to 7.4 µmol/L for 8 healthy volunteers. Chow et al. (2005) reported for a dose of 400, 800, or 1200 mg (0.9, 1.7, or 2.8 mmol) EGCG as GTE maximum EGCG levels of 0.3, 0.6, or 2 µmol/L, respectively, if the GTE was taken with a meal. If the GTE was taken on an empty stomach, there were markedly higher EGCG plasma levels of 1.7, 3.3, or 7.4 µmol/L, respectively. It was described here that the maximum EGCG plasma level was achieved after 1.3 to 4 hours. Thereafter, the plasma levels fell to the half- maximal values within 2 to 5 hours.

A 6-month phase I study of patients with tumours (Pisters et al. 2001) showed that a three times daily dose of 1 g GTE/m^2 (with 132 mg EGCG and 68 mg caffeine, total daily dose: 630 – 880 mg EGCG, 330 – 460 mg caffeine) is well tolerated. At higher doses, side effects were observed that were attributed to caffeine. In a phase I study of chronic lymphocytic leukaemia (CLL) patients (Shanafeldt et al. 2009), 400 to 2000 mg EGCG as caffeine-free GTE were taken twice daily over a period of at least 6 months. This study came to the conclusion that as a rule the EGCG doses used are well tolerated. In both studies, each dose was taken with a meal due to concerns about taking the substance under fasting conditions that were raised by the licensing commission.

The studies mentioned above of EGCG bioavailability in blood plasma showed that only a small amount of the ingested EGCG dose appears in the plasma. If there are concerns about the ingestion of EGCG on an empty stomach, then there may be alternative means by which the bioavailability of smaller doses of EGCG can be raised. We would like present here such a method.

The polyphenol EGCG is a strong antioxidant that may exert a protective effect in the gastrointestinal tract even before passage through the intestinal wall by reacting extensively with contents of the intestinal fluid (see Halliwell et al. 2005). Green et al. (2007) demonstrated via *in vitro* simulation experiments that a high proportion of EGCG is lost to oxidation in the fluid of the stomach and upper intestinal tract and that this loss can be avoided to a large extent by the use of vitamin C. Shoba et al. (1998) showed in humans that the plasma level of curcumin can be elevated 20-fold through the simultaneous ingestion of 20 mg piperine, the active component of black pepper. In animal experiments Lambert et al. (2004) showed that piperine also increased the bioavailability of EGCG. What would be the outcome of performing this experiment using EGCG, vitamin C, and piperine *in vivo* with human volunteers?

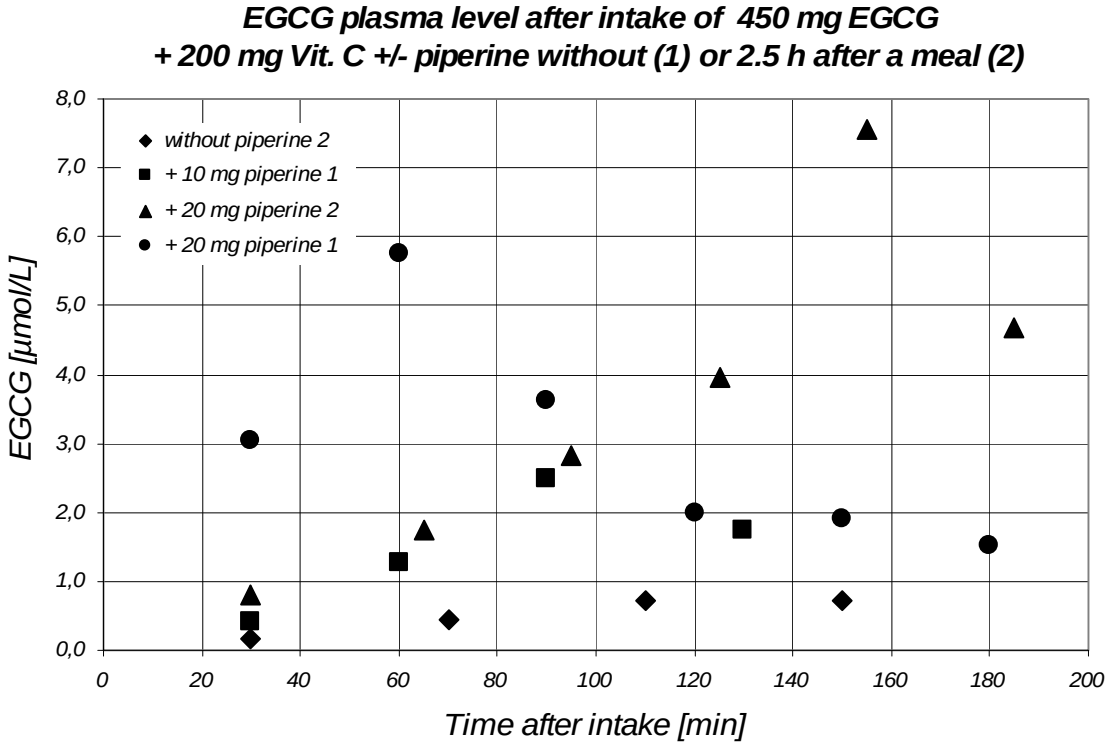
At the suggestion of WH, Rupert Schreiner (RS) set up a test to determine EGCG levels in human plasma. This test measured unchanged, free EGCG in plasma that is not bound to protein. Because EGCG at pH > 7 rapidly changes its original structure, binds to proteins, and becomes partially oxidized, the blood samples were

immediately transferred to an ice bath and the plasma or serum was separated from the cellular fraction by cold centrifugation and placed into small (1 ml) fractions containing vitamin C and an acidic phosphate buffer. The tubes were then immediately transferred to a freezer and shipped on dry ice as soon as possible for analysis.

Klaus Altland (KA) then obtained EGCG as caffeine-free GTE as well as piperine, both in capsule form. Descriptions of piperine can be found in a review by Srinivasan (2007) and in a comprehensive brochure from Sabinsa (see Mejeed et al. 1999). According to these sources, the average daily intake of an American is about 360 mg pepper or 18 - 32 mg piperine. The effective dose at which the bioavailability of nutrients is increased is listed at 28 – 56 mg for a 70 kg individual. The composition of the test dose was derived from this information: 450 mg EGCG plus 20 mg piperine plus 200 mg vitamin C. This test dose comprises the contents of about 1.5 litres green tea, 4 peppercorns, and less than half as much vitamin C (450 mg), which was on the daily menu of our stone-age ancestors. Over several years KA has taken 5 g fish oil per day with meal including the time during these experiments (see below).

The following figure shows the time course (November, 2008) of the EGCG plasma levels after ingestion of the test dose with and without piperine. The dose was taken on an empty stomach or 2.5 hours after breakfast. It can be seen that without piperine, a maximum plasma level of up to 0.7 µmol/L was attained. With 20 mg piperine, the maximum plasma level was 5.8 to 7.6 µmol/L, which is an 8- to 10-fold higher level of EGCG. This approaches the levels that were measured by Ullmann et al. (2003) for the dose of 1600 mg EGCG under fasting conditions. It should be noted that the maximum EGCG plasma level in the same individual can be achieved at different times after dose administration. It is possible that the time since the last meal plays an important role here.

The results of a test for intra-individual reproducibility of the effects of piperine on the EGCG plasma level are shown in the following table. The high consistency of the EGCG levels on various test days is noteworthy as is the dependence on the time between dose and meal intake (see bold-faced values).



Bioavailability of EGCG after repeated intake of EGCG in GTE, vitamin C, and piperine

Date	Time of Day			Test Dose			ECGC Plasma Level	
	Breakfast	Dose intake	Blood sampling	EGCG mg	Vit. C mg	Piperine mg	µg/L	µmol/L
5-Feb-09	07:00	09:53	12:27	450	200	20	1014	2,2
9-Feb-09	07:00	09:30	12:00	450	200	20	895	2
11-Feb-09	07:00	10:30	13:00	450	200	20	978	2,1
13-Feb-09	07:00	09:35	12:00	450	200	20	815	1,8
16-Feb-09	07:00	09:30	12:00	450	200	10	922	2
20-Feb-09	07:00	09:30	12:00	450	200	10	777	1,7
18-Feb-09	07:00	09:30	12:00	450	200	10	831	1,8
23-Feb-09	07:00	09:30	12:00	450	200	10	825	1,8
12-Mar-09	**10:00**	**07:00**	**09:30**	450	200	20	**2180**	**4,8**
13-Mar-09	**07:00**	**07:00**	**09:30**	450	200	20	**392**	**0,9**

Summary and Conclusions

Epidemiologic studies have shown that there is a relationship between green tea consumption and the risk for cancer and cardiovascular disease. Laboratory investigations have demonstrated that EGCG inhibits the formation of various types of amyloid and leads to the degradation of pre-existing amyloid. In both cases there is a positive correlation between EGCG dose and the desired effect. The experiment described above that WH performed on himself shows that the EGCG dose contained in 2 litres of green tea can lead to degradation of amyloid. The

experiments conducted by KA described here show that high plasma levels of EGCG can be achieved if the dose is taken together with vitamin C and piperine on an empty stomach. The effect of piperine is lost if EGCG is taken with a meal. Since piperine has been shown to increase the bioavailability of various drugs, there should be no complications if EGCG and piperine are taken on an empty stomach and other drugs are taken with meals. A higher continuous plasma level of EGCG should be more readily achievable through repeated intake of the test dose used here as compared with the level achieved through tea drinking alone. In this context, however, it should be mentioned that occasionally hepatotoxic effects have been observed after long-term intake of GTE (EFSA 2009). The experiments described here that the researchers performed on themselves should be repeated for a larger collective. As long as results of such studies are not available, attempts to reproduce our results should not be made without a physician's advice.

In December, 2008, WH changed over from drinking green tea to taking 450 mg EGCG, 20 mg piperine and 200 mg vitamin C twice daily. Several months after this change his tongue and cardiac septal wall thickened again. His kidneys worsened and EGCG plasma levels decreased to levels of < 0.1 µmol/L. He raised the dose to 900 mg EGCG, 20 mg piperine und 200 mg vitamin C three times a day since July, 2009. His tongue and heart walls returned to the norm again (see: http://www.hunstein-egcg.de). Later he replaced piperine by 1 ml of fish oil (Giunta et al. 2011). He has not experienced any side effects. His plasma EGCG level varied between 0.4 and 1.3 µmol/L. Presumably, there is a significant intraindividual variability of EGCG plasma levels associated with the expression of the disease. We conclude that the intake of GTE should be combined with a control of the EGCG plasma levels and that EGCG levels between 0.4 and 1.3 µmol/L 2 hours after intake are enough to result in a positive effect. Our experiments with GTE, vitamin C, and piperine demonstrate how these plasma levels of EGCG can be achieved.

Prof. Hunstein died on February 2012 at the age of 83 years.

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First version: November 2009; this version: February 2013

Source:
http://www.uni-giessen.de/cms/fbz/fb11/institute/klinik/humangenetik/Downloads

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