

## Oral treatment with L-lysine and L-arginine reduces anxiety and basal cortisol levels in healthy humans

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### ABSTRACT

Dietary supplementation with an essential amino acid L-lysine has been shown to reduce chronic anxiety in humans with low dietary intake of L-lysine. A combination of L-lysine and L-arginine has been documented to normalize hormonal stress responses in humans with high trait anxiety. The present study was carried out in one hundred eight healthy Japanese adults. The aim of study was to find out whether a week-long oral treatment with L-lysine (2.64 g per day) and L-arginine (2.64 g per day) reduces trait and stress-induced state anxiety and basal levels of stress hormones. We confirmed that, without regard to gender, the amino acid treatment significantly reduced both trait anxiety and state anxiety induced by cognitive stress battery. In addition, we found that the treatment with L-lysine and L-arginine decreased the basal levels of salivary cortisol and chromogranin-A (a salivary marker of the sympatho-adrenal system) in male subjects. These results of this double-blind, placebo controlled and randomized study confirm the previous findings in humans and animals and point to a combination of L-lysine and L-arginine as a potentially useful dietary intervention in otherwise healthy humans with high subjective levels of mental stress and anxiety.

Chronic mental stress or high basal level of stress hormones contribute to the development of peripheral diseases (15, 28) and clinical anxiety (19, 24, 29). Coping with mental stress depends on genetic and exogenous parameters as well as dietary supply of essential micronutrients, such as essential amino acids (7, 8, 11, 35). The two amino acids (L-tryptophan and L-tyrosine), which are precursors of the brain neurotransmitters have been most frequently studied in respect to stress modulation (1, 5, 8, 16, 17, 25), but the results were inconsistent.

Recent results have indicated that the essential

amino acid L-lysine (Lys), which is not a direct neurotransmitters precursor, reduces anxiety (34) and normalizes stress-induced hormonal responses in otherwise healthy subjects with relatively high perceived anxiety (11) and when applied with L-arginine (Arg), blocks stress-induced pathologies in laboratory and farm animals (31, 32, 37). Physiological mechanism underlining the above effects is probably coupled to Lys acting as a partial serotonin receptor 4 antagonist (9, 33) and simultaneously as a partial benzodiazepine agonist (2, 3).

Herein, we have extended the research work on Lys and Arg by conducting a double-blind, randomized, placebo-controlled trial which evaluated the effectiveness of their combination (henceforth, Lys/Arg) in reducing trait and state anxiety and improving an acute response to a cognitive stress battery in healthy adult subjects. Salivary levels of the hypoth-

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alomo-pituitary-adrenal hormone cortisol and the sympathetic system marker, chromogranin-A (13, 21, 22, 38), were used as objective measures of stress response. The standard Japanese version of the State Trait Anxiety Inventory (STAI, 36) served as a subjective measure of stress and anxiety perception.

## MATERIALS AND METHODS

One hundred and eight healthy participants (54 males and 54 females, aged between 22 and 59 years) were recruited through local advertisements (Kawasaki, Japan). All participants were considered for selection if they signed an informed consent, were healthy, non-medicated and had no known personal history of psychiatric disorders as determined by clinical interviews. No other restrictions on the daily routine, dietary or smoking habits were placed on the subjects, although it is probable that participating subjects were concerned with the impact of daily stress upon their daily routine.

The study was a double-blind, placebo-controlled randomized design, in which all subjects were tested under a single treatment regime. Before the start of the treatment, all subjects responded at 10:00 am to the trait part of the STAI (36). The basal salivary levels of cortisol and chromogranin-A were also obtained, as described before (14, 21, 30, 39). Briefly, cotton wads (Sarstedt Co., Ltd., Numbrecht, Germany) held for 2 min in the mouth of the subjects were used for collection of saliva, which was then extracted by centrifuging (3,000 rpm, 15 min) and stored at  $-80^{\circ}\text{C}$  until being assayed.

The salivary cortisol and chromogranin-A were analyzed by Yanaihara Institute Inc. (Shizuoka, Japan) using previously described methods (14, 34, 39). Computerized randomization of the subjects was based on the results of the trait inventory. Immediately following the randomization, subjects assigned to the test group (Lys/Arg group) received hard capsules (Aliment Co., Ltd, Tokyo, Japan) containing L-lysine HCl and L-arginine (Ajinomoto Co., Inc., Tokyo, Japan) and were instructed to ingest the capsules twice daily (at breakfast and dinner) during seven consecutive days. The dose of both amino acids was adjusted in such a way that the subjects ingested both L-lysine HCl and L-Arginine at  $1.32\text{ g} \times 2/\text{day}$ . The subjects assigned to placebo (placebo group) received hard capsules containing tapioca starch ( $3.00\text{ g} \times 2/\text{day}$ ). The test and placebo capsules were matching in color, odor, and shape and were without any taste properties. Ran-

dom laboratory tests of the capsules using high-performance liquid chromatography assay confirmed the required content of the amino acids.

On the seventh day of the treatment, subjects were summoned at 9:40 in a large ventilated room for re-evaluation of salivary values of both chromogranin-A and cortisol. At 10:00, all subjects again responded to the STAI inventory. Two subscales, state and trait, of the inventory were applied for the evaluation of state and trait anxiety, respectively. Thereafter, the subjects were subjected to a 20-min-long cognitive stress battery, during which loud metronome sound was presented from ceiling speakers (frequency was gradually increased from 80 to 120 beats per min). Immediately following the cognitive battery test, salivary chromogranin-A was re-measured and the subjects responded to the state part of STAI inventory. To reach again the pre-stress (basal) values of hormonal markers, the subjects were allowed to view a relaxing video projection for 20 min. At the end of the projection, the two salivary markers (cortisol and chromogranin-A) were again obtained. Body weight and abdominal fat were measured before and after capsule treatment. The study protocol was approved by the Ajinomoto Ethical Committee.

Comparisons within multiple groups were performed using a two-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P values  $< 0.05$  were considered statistically significant. Values are expressed as means  $\pm$  SEM (the values with different superscript letters differ significantly).

## RESULTS

One hundred eight subjects were randomized, and one hundred seven subjects received the tested capsules. Among the fifty-three subjects who received Lys/Arg-containing capsules, three subjects withdrew from the trial, and fifty subjects (94.3%) finished the trial correspondingly to the protocol. No treatment-related cause for trial withdrawal and no side effects were reported.

Trait part of the STAI inventory was obtained at the same time of day (Tuesday, 10:00 am) before, and immediately after the week-long capsule treatment. The results are shown in upper part of Table 1. Lys/Arg treatment significantly reduced trait anxiety when compared to placebo treatment. The effect was comparable in men and women, and therefore summarized results for all subjects are shown. State part of the STAI inventory (lower part

**Table 1** STAI score (Mean  $\pm$  SEM)

Trait part of STAI		
	before treatment	after treatment ( <i>before stress</i> )
Placebo (N = 54)	52.00 $\pm$ 1.13 <sup>a</sup>	51.36 $\pm$ 1.10 <sup>a</sup>
Lys/Arg (N = 50)	53.06 $\pm$ 1.12 <sup>a</sup>	47.26 $\pm$ 1.11 <sup>b</sup>

  

State part of STAI		
	after treatment	
	<i>basal</i>	<i>after stress</i>
Placebo (N = 54)	49.46 $\pm$ 1.01 <sup>b</sup>	54.17 $\pm$ 1.22 <sup>a</sup>
Lys/Arg (N = 50)	47.00 $\pm$ 0.95 <sup>b</sup>	47.38 $\pm$ 1.05 <sup>b</sup>

The values with different superscript letters differ significantly at  $P < 0.05$  by Duncan's multiple range test.

**Table 2** Salivary cortisol [ $\mu\text{g/dL}$ ]

Males (n = 25, 27 $\pm$ SEM)				
	before treatment		after treatment	
	<i>basal</i>	<i>basal</i>	<i>20 min after stress</i>	
Placebo	0.61 $\pm$ 0.05 <sup>a</sup>	0.62 $\pm$ 0.09 <sup>a</sup>	0.64 $\pm$ 0.05 <sup>a</sup>	
Lys/Arg	0.56 $\pm$ 0.06 <sup>a</sup>	0.46 $\pm$ 0.06 <sup>b</sup>	0.72 $\pm$ 0.09 <sup>a</sup>	

  

Females (n = 25, 27 $\pm$ SEM)				
	before treatment		after treatment	
	<i>basal</i>	<i>basal</i>	<i>20 min after stress</i>	
Placebo	0.44 $\pm$ 0.04	0.42 $\pm$ 0.05	0.46 $\pm$ 0.05	
Lys/Arg	0.43 $\pm$ 0.04	0.38 $\pm$ 0.05	0.48 $\pm$ 0.05	

The values with different superscript letters differ significantly at  $P < 0.05$  by Duncan's multiple range test.

of Table 1) was obtained from each tested subject after the capsule treatment (immediately before respectively after the cognitive stress battery test). The stress battery increased state anxiety in placebo-treated subject by approximately 9.5% and this increase was significantly blunted by Lys/Arg treatment.

Salivary cortisol was measured in all subjects three times; before the capsule treatment and twice after the treatment (Table 2). The results differed between the genders; while Lys/Arg treatment reduced basal saliva cortisol levels in males, no differences were obtained in females. Cognitive stress battery enhanced salivary cortisol in males treated with Lys/Arg but not in placebo controls (Table 2). The values of cortisol measured in Lys/Arg group at 20 min post-stress were comparable to those measured in placebos. No stress effect on salivary cortisol was observed in females.

Chromogranin-A values varied substantially among the subjects and therefore the results are expressed in relative values (Table 3). At the end of the cap-

sule treatment, salivary chromogranin-A was significantly lower in the males treated with Lys/Arg as compared to those treated with placebo. The cognitive battery stress enhanced chromogranin-A values in Lys/Arg treated male subjects, but not in placebos. Chromogranin A values measured 20 min after stress exposure were comparable to pre-stress values in Lys/Arg treated subjects, while no change, when compared to pre-stress or stress values, was seen in the placebo group. Neither cognitive battery stress nor the treatment affected the salivary chromogranin-A levels in females. The treatment with Lys/Arg had no significant effect on body weight or abdominal fat ratio (data not shown).

## DISCUSSION

A week-long treatment with Lys/Arg decreased trait anxiety, blocked stress-induced state anxiety in both genders, and reduced basal values of the salivary cortisol and chromogranin-A in males. Cortisol is a hormonal marker of the hypothalamo-pituitary-adre-

**Table 3** Normalized values of salivary chromogranin-A [%]

Males (n = 25, 27 ± SEM)				
	before treatment		after treatment	
	basal	basal	immediately after stress	20 min after stress
Placebo	100.00	134.06 ± 22.38 <sup>a</sup>	124.31 ± 19.05 <sup>a</sup>	125.81 ± 20.58 <sup>a</sup>
Lys/Arg	100.00	85.23 ± 10.28 <sup>b</sup>	143.41 ± 29.73 <sup>a</sup>	96.07 ± 12.65 <sup>a,b</sup>

  

Females (n = 25, 27 ± SEM)				
	before treatment		after treatment	
	basal	basal	immediately after stress	20 min after stress
Placebo	100.00	120.55 ± 18.41	128.48 ± 23.03	139.13 ± 25.69
Lys/Arg	100.00	116.45 ± 28.49	119.30 ± 22.77	139.09 ± 35.64

The values with different superscript letters differ significantly at  $P < 0.05$  by Duncan's multiple range test.

nal axis. Chromogranin A is a soluble protein in adrenergic neurons. Although histochemical studies (26, 27) revealed that the predominant source of salivary chromogranin A is salivary glands themselves, this protein directly reflects the sympathetic tone and sympathetic stress response (13, 21, 22, 38). The results agree with previously obtained human data (34) and, at hormonal level, correspond to the results reported in pigs (37). While all subjects tested in the trial were found healthy by a physician, it was assumed that most of the participating subjects were concerned with the level of mental stress in their daily lives.

A high basal cortisol and an increased basal sympathetic tone lead to psychological pathologies and blunt normal responsiveness of both the hypothalamo-pituitary-adrenal and the sympathetic axes to stress exposure (4, 6, 10, 12, 43) as seen in clinical anxiety (6). Thus, we postulate that the reduction of basal cortisol and chromogranin-A by Lys/Arg contributed to a lower anxiety and to an improved responsiveness of both axes to acute mental stress in the Lys/Arg-treated males, as attested by the stress-specific responses of cortisol and chromogranin-A. It needs to be noted that while Lys/Arg was anxiolytic in both genders, the basal values of cortisol and chromogranin-A declined only in males, and therefore the declines were not the only physiological mechanisms underlining the anxiolytic effect of Lys/Arg. There is still insufficient clinical evidence to enable full reasoning on the gender difference in hormonal responses, but the reasons could be three-fold: low salivary values measured in females (about 30% lower when compared to males), co-influence of menstrual cycle which was not measured, and gender-specific hormonal reflection of mental stress (14, 34).

Due to technical reasons, we were not able to

measure salivary cortisol level later than 20 min post-stress, and therefore could not observe a more detailed time-response. Because the stress-response of the sympathetic system is rapid (11), all three phases of the sympathetic stress response (pre-stress basal, stress level and post-stress basal) were observed within the timeframe of the trial. In the Lys/Arg group, chromogranin-A specifically reacted to the cognitive stress and returned to basal levels 20 min after the stress offset, while the values measured in placebo males remained high before, during and after the cognitive stress battery.

Discussion on the mechanisms on anti-anxiety effects of Lys/Arg is complicated as no information on the dietary habits of the evaluated subjects is available. The subjects were instructed to preserve their normal dietary life-style, implying that approximately 5.0–6.0 grams of both Lys and Arg were ingested daily. Therefore, the supplemental treatment increased the intake of both amino acids by approximately 50%. This substantial increase might have not only lowered basal cortisol and curbed sympathetic tone, but also triggered previously-described pharmacological-like effects in the gut and the brain through the benzodiazepine, serotonin (2, 3, 9, 35) or amino acid-specific (42) receptors. A positive, stress-dependent influence on synthesis of proteins involved in stress response cannot be excluded, because a recent human study revealed that mental fatigue triggered by a single cognitive test significantly decreased plasma lysine levels. The decrease persisted until as late as 24 h post-test (18).

In conclusion, this double-blind, randomized, placebo-controlled trial documented a significant decrease in both the long-term and stress-induced anxiety in healthy adults treated for one week with orally given Lys and Arg. Because the safety profiles of dietary Lys and Arg are well established (23,

40, 41), their combination may provide a useful dietary intervention in humans with high perceived stress and anxiety.

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